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U.S. PTO

Express Mail Label No.

# UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.  
14917.1.1

Total Pages in this Submission  
109

**TO THE ASSISTANT COMMISSIONER FOR PATENTS**  
**Box Patent Application**  
**Washington, D.C. 20231**

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for invention entitled:

**INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATHOGRAPHY SYSTEM AND METHOD**

and invented by:

**James E. Moon, Gary A. Schultz, Thomas N. Corso, Timothy J. Davis, Gregory J. Galvin, and Stephen Lowes**

**If a CONTINUATION APPLICATION, check appropriate box and supply the requisite information:**

☐ Continuation    ☒ Divisional    ☐ Continuation-in-part (CIP) of prior application No.: 09/156,507

Which is a:

☐ Continuation    ☐ Divisional    ☐ Continuation-in-part (CIP) of prior application No.: \_\_\_\_\_

Which is a:

☐ Continuation    ☐ Divisional    ☐ Continuation-in-part (CIP) of prior application No.: \_\_\_\_\_

Enclosed are:

## Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 62 pages and including the following:
  - a. ☒ Descriptive Title of the Invention
  - b. ☒ Cross References to Related Applications (if applicable)
  - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
  - d. ☐ Reference to Microfiche Appendix (if applicable)
  - e. ☒ Background of the Invention
  - f. ☒ Brief Summary of the Invention
  - g. ☒ Brief Description of the Drawings (if drawings filed)
  - h. ☒ Detailed Description
  - i. ☒ Claim(s) as Classified Below
  - j. ☒ Abstract of the Disclosure

U.S. PTO  
09/698329

10/27/00

# UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.

14917.1.1

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109

## Application Elements (Continued)

3. ☒ Drawing(s) (when necessary as prescribed by 35 USC 113)
- a. ☐ Formal      b. ☒ Informal      Number of Sheets 26
4. ☒ Oath or Declaration
- a. ☐ Newly executed (original or copy)      ☐ Unexecuted
- b. ☒ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)
- c. ☒ With Power of Attorney      ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting inventor(s) named in the prior application,  
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☒ Incorporation By Reference (usable if Box 4b is checked)  
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche
7. ☐ Genetic Sequence Submission (if applicable, all must be included)
- a. ☐ Paper Copy
- b. ☐ Computer Readable Copy
- c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

## Accompanying Application Parts

8. ☐ Assignment Papers (cover sheet & documents)
9. ☐ 37 CFR 3.73(b) Statement (when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement/PTO-1449      ☐ Copies of IDS Citations
12. ☒ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing
- ☐ First Class      ☒ Express Mail (Specify Label No.): EL675131054US

**UTILITY PATENT APPLICATION TRANSMITTAL  
(Small Entity)**

*(Only for new nonprovisional applications under 37 CFR 1.53(b))*

Docket No.  
14917.1.1

Total Pages in this Submission  
109

**Accompanying Application Parts (Continued)**

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. ☒ Small Entity Statement(s) - Specify Number of Statements Submitted: 2
17. ☒ Additional Enclosures *(please identify below)*:

Associate Power of Attorney  
Assignment Documents (2 sets - 8 pages and 6 pages)  
Credit Card Payment Form Authorizing \$355.00

**Request That Application Not Be Published Pursuant To 35 U.S.C. 122(b)(2)**

18. ☐ Pursuant to 35 U.S.C. 122(b)(2), Applicant hereby requests that this patent application not be published pursuant to 35 U.S.C. 122(b)(1). Applicant hereby certifies that the invention disclosed in this application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication of applications 18 months after filing of the application.

**Warning**

***An applicant who makes a request not to publish, but who subsequently files in a foreign country or under a multilateral international agreement specified in 35 U.S.C. 122(b)(2)(B)(i), must notify the Director of such filing not later than 45 days after the date of the filing of such foreign or international application. A failure of the applicant to provide such notice within the prescribed period shall result in the application being regarded as abandoned, unless it is shown to the satisfaction of the Director that the delay in submitting the notice was unintentional.***

**UTILITY PATENT APPLICATION TRANSMITTAL**  
**(Small Entity)**

*(Only for new nonprovisional applications under 37 CFR 1.53(b))*

Docket No.  
14917.1.1

Total Pages in this Submission  
103

**Fee Calculation and Transmittal**

**CLAIMS AS FILED**

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	1	- 20 =	0	x \$9.00	\$0.00
Indep. Claims	1	- 3 =	0	x \$40.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$355.00
OTHER FEE (specify purpose) _____					\$0.00
TOTAL FILING FEE					\$355.00

- ☐ A check in the amount of \_\_\_\_\_ to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. **23-3178** as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of **\$355.00** as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: October 27, 2000

  
Signature

David O. Seeley  
Attorney for Applicants  
Registration No. 30,148



**022913**

DOS:cm  
CC:

PATENT TRADEMARK OFFICE

SMALL BUSINESS  
Docket No.: 200701/1030

Applicant or Patentee : James E. Moon, Gary A. Schultz, Thomas N. Corso,  
Timothy J. Davis, Gregory J. Galvin, Stephen Lowes  
Serial or Patent No. : 09/156,507  
Filed or Issued : September 17, 1998  
For : INTEGRATED MONOLITHIC MICROFABRICATED  
ELECTROSPRAY AND LIQUID CHROMATOGRAPHY  
SYSTEM AND METHOD

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act on behalf of the concern  
identified below:

NAME OF CONCERN : Kionix, Inc.  
ADDRESS OF CONCERN : 22 Thornwood Drive  
Ithaca, New York 14850

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled **INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY SYSTEM AND METHOD** by inventors James E. Moon, Gary A. Schultz, Thomas N. Corso, Timothy J. Davis, Gregory J. Galvin, Stephen Lowes described in

- ☐ the specification filed herewith  
☒ U.S. Patent Application Serial No.: 09/156,507  
Filed: September 17, 1998  
☐ U.S. Patent No.:  
Issued:

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). \*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME : Advanced Bioanalytical Services, Inc.  
ADDRESS : 15 Catherwood Road, Ithaca, New York 14850

☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

NAME :  
ADDRESS :

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING : Gregory J. Galvin  
TITLE OF PERSON OTHER THAN OWNER : President  
ADDRESS OF PERSON SIGNING : 22 Thornwood Drive  
Ithaca, New York 14850

SIGNATURE: \_\_\_\_\_

DATE: 3/11/99

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. § 1.10

JC920 U.S. PRO  
09/698329  
10/27/00

"Express Mail" Mailing Label No.: EL675131054US

Date of Deposit: October 27, 2000

I hereby certify that the following documents are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above in an envelope addressed to: Box PATENT APPLICATION, Assistant Commissioner for Patents, Washington, D.C. 20231:

- Divisional Patent Application in the names of James E. Moon, Gary A. Schultz, Thomas N. Corso, Timothy J. Davis; Gregory J. Galvin and Stephen Lowes for INTEGRATED MONOLITHIC MICROFAABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY SYSTEM AND METHOD (62 pages)
- Informal Drawings (26 pages)
- Copy of the Declaration, Power of Attorney and Petition (2 - 3 pages each)
- Copy of Small Entity Statements (2 - 2 pages each)
- Copy of Assignment Documents (2 - 8 pages and 6 pages)
- Associate Power of Attorney (2 pages)
- Preliminary Amendment
- Transmittal Letter (4 pages)
- Credit Card Payment Form authorizing \$355.00 fee
- Certificate of Express Mail Label No. EL675131054US
- Postcard

Dated this 27th day of October 2000.

Respectfully submitted,



David O. Seeley  
Attorney for Applicant  
Registration No. 30,148



022913

SMALL BUSINESS  
Docket No.: 200701/1030

Applicant or Patentee : James E. Moon, Gary A. Schultz, Thomas N. Corso,  
Timothy J. Davis, Gregory J. Galvin, Stephen Lowes  
Serial or Patent No. : 09/156,507  
Filed or Issued : September 17, 1998  
For : INTEGRATED MONOLITHIC MICROFABRICATED  
ELECTROSPRAY AND LIQUID CHROMATOGRAPHY  
SYSTEM AND METHOD

JC920 U.S. PTO  
09/698329  
10/27/00

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act on behalf of the concern  
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I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled  
**INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY SYSTEM AND METHOD** by inventors James E. Moon, Gary A. Schultz, Thomas N. Corso, Timothy J. Davis, Gregory J. Galvin, Stephen Lowes described in

- ☐ the specification filed herewith  
☒ U.S. Patent Application Serial No.: 09/156,507  
Filed: September 17, 1998  
☐ U.S. Patent No.:  
Issued:

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). \*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME : Kionix, Inc.  
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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING : **Thomas R. Kurz**  
TITLE OF PERSON OTHER THAN OWNER : **General Manager**  
ADDRESS OF PERSON SIGNING : **15 Catherwood Road**  
**Ithaca, New York 14850**

SIGNATURE: Thomas R. King DATE: MARCH 12, 1999

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: )  
 )  
 J. Moon et al. )  
 )  
 Divisional of )  
 Serial No.: 09/156,507 ) Art Unit  
 ) 1741  
 Filed: September 17, 1998 )  
 )  
 For: INTEGRATED MONOLITHIC MICROFABRICATED )  
 ELECTROSPRAY AND LIQUID )  
 CHROMATOGRAPHY SYSTEM AND METHOD )  
 )  
 Examiner: Thao Tran )

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Applicant hereby cancels the following claims as originally filed: Claims 1-9; 11-12; 13-16;  
17; 18-20; 21 and 22 without prejudice.

**REMARKS**

This is a divisional patent application directed to Group II, consisting of Claim 10, as  
established in an Office Action dated the 26<sup>th</sup> of September, 2000 in which the Examiner established  
a Restriction Requirement.

PATENT APPLICATION

Docket No.: 14917.1.1

Dated this 27th day of October, 2000.

Respectfully submitted,



David O. Seeley  
Attorney for Applicants  
Reg. No. 30,148

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[illegible]

WORKMAN, NYDEGGER &amp; SEELEY

A PROFESSIONAL CORPORATION  
ATTORNEYS AT LAW  
1000 EAGLE GATE TOWER  
60 EAST SOUTH TEMPLE  
SALT LAKE CITY, UTAH 84111

## of

Gary A. Schultz

Timothy J. Davis

Gregory J. Galvin

and

Stephen Lowes

# INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY SYSTEM AND METHOD

1 This application is a divisional of U.S. Application Serial No. 09/156,507 filed  
2 September 17, 1998.

### 3 FIELD OF THE INVENTION

4 The present invention relates generally to an integrated miniaturized chemical  
5 analysis system fabricated using microelectromechanical systems (MEMS) technology. In  
6 particular, the present invention relates to an integrated monolithic microfabricated  
7 electrospray and liquid chromatography device. This achieves a significant advantage in  
8 terms of high-throughput analysis by mass spectrometry, as used, for example, in drug  
9 discovery, in comparison to a conventional system.

### 10 BACKGROUND OF THE INVENTION

11 New developments in drug discovery and development are creating new demands  
12 on analytical techniques. For example, combinatorial chemistry is often employed to  
13 discover new lead compounds, or to create variations of a lead compound. Combinatorial  
14 chemistry techniques can generate thousands or millions of compounds (combinatorial  
15 libraries) in a relatively short time (on the order of days to weeks). Testing such a large  
16 number of compounds for biological activity in a timely and efficient manner requires high-  
17 throughput screening methods which allow rapid evaluation of the characteristics of each  
18 candidate compound.

19 The compounds in combinatorial libraries are often tested simultaneously against  
20 a molecular target. For example, an enzyme assay employing a colorimetric measurement  
21 may be run in a 96-well plate. An aliquot of enzyme in each well is combined with tens or  
22 hundreds of compounds. An effective enzyme inhibitor will prevent development of color  
23 due to the normal enzyme reaction, allowing for rapid spectroscopic (or visual) evaluation  
24 of assay results. If ten compounds are present in each well, 960 compounds can be screened  
25 in the entire plate, and one hundred thousand compounds can be screened in 105 plates,  
26 allowing for rapid and automated testing of the compounds.

Often, however, determination of which compounds are present in certain portions  
of a combinatorial library is difficult, due to the manner of synthesis of the library. For  
example, the "split-and-pool" method of random peptide synthesis in U.S. Pat. No.  
5,182,366, describes a way of creating a peptide library where each resin bead carries a  
unique peptide sequence. Placing ten beads in each well of a 96-well plate, followed by  
cleavage of the peptides from the beads and removal of the cleavage solution, would result

1 in ten (or fewer) peptides in each well of the plate. Enzyme assays could then be carried out  
2 in the plate wells, allowing 100,000 peptides to be screened in 105 plates. However, the  
identity of the peptides would not be known, requiring analysis of the contents of each well.

3 The peptides could be analyzed by removing a portion of solution from each well  
4 and injecting the contents into a separation device such as liquid chromatography or capillary  
5 electrophoresis instrument coupled to a mass spectrometer. Assuming that such a method  
6 would take approximately 5 minutes per analysis, it would require over a month to analyze  
the contents of 105 96-well plates, assuming the method was fully automated and operating  
7 24 hours a day.

8 This example illustrates the critical need for a method for rapid analysis of large  
9 numbers of compounds or complex mixtures of compounds, particularly in the context of  
high-throughput screening. Techniques for generating large numbers of compounds, for  
10 example through combinatorial chemistry, have been established. High-throughput  
screening methods are under development for a wide variety of targets, and some types of  
11 screens, such as the colorimetric enzyme assay described above and ELISA (enzyme linked  
12 immunosorbent assay) technology, are well-established. As indicated in the example above,  
13 a bottleneck often occurs at the stage where multiple mixtures of compounds, or even  
multiple individual compounds, must be characterized.

14 This need is further underscored when current developments in molecular  
15 biotechnology are considered. Enormous amounts of genetic sequence data are being  
16 generated through new DNA sequencing methods. This wealth of new information is  
generating new insights into the mechanism of disease processes. In particular, the  
17 burgeoning field of genomics has allowed rapid identification of new targets for drug  
development efforts. Determination of genetic variations between individuals has opened  
18 up the possibility of targeting drugs to individuals based on the individual's particular genetic  
19 profile. Testing for cytotoxicity, specificity, and other pharmaceutical characteristics could  
20 be carried out in high-throughput assays instead of expensive animal testing and clinical  
21 trials. Detailed characterization of a potential drug or lead compound early in the drug  
22 development process thus has the potential for significant savings both in time and expense.

23 Development of viable screening methods for these new targets will often depend  
24 on the availability of rapid separation and analysis techniques for analyzing the results of  
assays. For example, an assay for potential toxic metabolites of a candidate drug would need  
25 to identify both the candidate drug and the metabolites of that candidate. An assay for  
26

1 specificity would need to identify compounds which bind differentially to two molecular  
2 targets such as a viral protease and a mammalian protease.

3 It would therefore be advantageous to provide a method for efficient proteomic  
4 screening in order to obtain the pharmacokinetic profile of a drug early in the evaluation  
5 process. An understanding of how a new compound is absorbed in the body and how it is  
6 metabolized can enable prediction of the likelihood for an increased therapeutic effect or lack  
7 thereof.

8 Given the enormous number of new compounds that are being generated daily, an  
9 improved system for identifying molecules of potential therapeutic value for drug discovery  
10 is also critically needed.

11 It also would be desirable to provide rapid sequential analysis and identification of  
12 compounds which interact with a gene or gene product that plays a role in a disease of  
13 interest. Rapid sequential analysis can overcome the bottleneck of inefficient and time-  
14 consuming serial (one-by-one) analysis of compounds.

15 Accordingly, there is a critical need for high-throughput screening and identification  
16 of compound-target reactions in order to identify potential drug candidates.

17 Microchip-based separation devices have been developed for rapid analysis of large  
18 numbers of samples. Compared to other conventional separation devices, these microchip-  
19 based separation devices have higher sample throughput, reduced sample and reagent  
20 consumption and reduced chemical waste. The liquid flow rates for microchip-based  
21 separation devices range from approximately 1-300 nanoliters (nL) per minute for most  
22 applications.

23 Examples of microchip-based separation devices include those for capillary  
24 electrophoresis (CE), capillary electrochromatography (CEC) and high-performance liquid  
25 chromatography (HPLC). See Harrison *et al.*, Science 1993, 261, 859-897; Jacobson *et al.*  
26 Anal. Chem. 1994, 66, 1114-1118; and Jacobson *et al.* Anal. Chem. 1994, 66, 2369-2373.  
Such separation devices are capable of fast analyses and provide improved precision and  
reliability compared to other conventional analytical instruments.

Liquid chromatography (LC) is a well-established analytical method for separating  
components of a fluid for subsequent analysis and/or identification. Traditionally, liquid  
chromatography utilizes a separation column, such as a cylindrical tube, filled with tightly  
packed beads, gel or other appropriate particulate material to provide a large surface area.  
The large surface area facilitates fluid interactions with the particulate material, and the  
tightly packed, random spacing of the particulate material forces the liquid to travel over a

1 much longer effective path than the length of the column. In particular, the components of  
2 the fluid interact with the stationary phase (the particles in the liquid chromatography  
3 column) as well as the mobile phase (the liquid eluent flowing through the liquid  
4 chromatography column) based on the partition coefficients for each of the components. The  
5 partition coefficient is defined as the ratio of the time an analyte spends interacting with  
6 the stationary phase to the time spent interacting with the mobile phase. The longer an  
7 analyte interacts with the stationary phase, the higher the partition coefficient and the longer  
8 the analyte is retained on the liquid chromatography column. The components may be  
9 detected spectroscopically after elution from the liquid chromatography column by coupling  
10 the exit of the column to a post-column detector.

11 Spectroscopic detectors rely on a change in refractive index, ultraviolet and/or  
12 visible light absorption, or fluorescence after excitation with a suitable wavelength to detect  
13 the separated components. Alternatively, the separated components may be passed from the  
14 liquid chromatography column into other types of analytical instruments for analysis. The  
15 analysis outcome depends upon the sequenced arrival of the components separated by the  
16 liquid chromatography column and is therefore time-dependent.

17 The length of liquid transport from the liquid chromatography column to the analysis  
18 instrument such as the detector is preferably minimized in order to minimize diffusion and  
19 thereby maximize the separation efficiency and analysis sensitivity. The transport length is  
20 referred to as the dead volume or extra-column volume.

21 Capillary electrophoresis is a technique that utilizes the electrophoretic nature of  
22 molecules and/or the electroosmotic flow of fluids in small capillary tubes to separate  
23 components of a fluid. Typically a fused silica capillary of 100  $\mu\text{m}$  inner diameter or less is  
24 filled with a buffer solution containing an electrolyte. Each end of the capillary is placed in  
25 a separate fluidic reservoir containing a buffer electrolyte.

26 A potential voltage is placed in one of the buffer reservoirs and a second potential  
voltage is placed in the other buffer reservoir. Positively and negatively charged species will  
migrate in opposite directions through the capillary under the influence of the electric field  
established by the two potential voltages applied to the buffer reservoirs. Electroosmotic  
flow is defined as the fluid flow along the walls of a capillary due to the migration of charged  
species from the buffer solution. Some molecules exist as charged species when in solution  
and will migrate through the capillary based on the charge-to-mass ratio of the molecular  
species. This migration is defined as electrophoretic mobility. The electroosmotic flow and  
the electrophoretic mobility of each component of a fluid determine the overall migration for



1 each fluidic component. The fluid flow profile resulting from electroosmotic flow is flat due  
2 to the reduction in frictional drag along the walls of the separation channel. This results in  
3 improved separation efficiency over liquid chromatography where the flow profile is  
4 parabolic resulting from pressure driven flow.

5 Capillary electrochromatography is a hybrid technique which utilizes the electrically  
6 driven flow characteristics of electrophoretic separation methods within capillary columns  
7 packed with a solid stationary phase typical of liquid chromatography. It couples the  
8 separation power of reversed-phase liquid chromatography with the high efficiencies of  
9 capillary electrophoresis. Higher efficiencies are obtainable for capillary electro-  
10 chromatography separations over liquid chromatography because the flow profile resulting  
11 from electroosmotic flow is flat due to the reduction in frictional drag along the walls of the  
12 separation channel when compared to the parabolic flow profile resulting from pressure  
13 driven flows. Furthermore, smaller particle sizes can be used in capillary  
14 electrochromatography than in liquid chromatography because no back pressure is generated  
15 by electroosmotic flow. In contrast to electrophoresis, capillary electrochromatography is  
16 capable of separating neutral molecules due to analyte partitioning between the stationary and  
17 mobile phases of the column particles using a liquid chromatography separation mechanism.

18 The separated product of such separation devices may be introduced as the liquid  
19 sample to a device that is used to produce electrospray ionization. The electrospray device  
20 may be interfaced to an atmospheric pressure ionization mass spectrometer (API-MS) for  
21 analysis of the electrosprayed fluid.

22 A schematic of an electrospray system 50 is shown in FIG. 1. An electrospray is  
23 produced when a sufficient electrical potential difference  $V_{\text{spray}}$  is applied between a  
24 conductive or partly conductive fluid exiting a capillary orifice and an electrode so as to  
25 generate a concentration of electric field lines emanating from the tip or end of a capillary  
26 52 of an electrospray device. When a positive voltage  $V_{\text{spray}}$  is applied to the tip of the  
capillary relative to an extracting electrode 54, such as one provided at the ion-sampling  
orifice to the mass spectrometer, the electric field causes positively-charged ions in the fluid  
to migrate to the surface of the fluid at the tip of the capillary. When a negative voltage  $V_{\text{spray}}$   
is applied to the tip of the capillary relative to an extracting electrode 54, such as one  
provided at the ion-sampling orifice to the mass spectrometer, the electric field causes  
negatively-charged ions in the fluid to migrate to the surface of the fluid at the tip of the  
capillary.

1 When the repulsion force of the solvated ions exceeds the surface tension of the fluid  
2 sample being electrosprayed, a volume of the fluid sample is pulled into the shape of a cone,  
3 known as a Taylor cone 56 which extends from the tip of the capillary. Small charged  
4 droplets 58 are formed from the tip of the Taylor cone 56 and are drawn toward the  
5 extracting electrode 54. This phenomenon has been described, for example, by Dole et al.,  
6 *Chem. Phys.* 1968, 49, 2240 and Yamashita and Fenn, *J. Phys. Chem.* 1984, 88, 4451. The  
7 potential voltage required to initiate an electrospray is dependent on the surface tension of  
8 the solution as described by, for example, Smith, *IEEE Trans. Ind. App.* 1986, IA-22, 527-  
9 535. Typically, the electric field is on the order of approximately  $10^6$  V/m. The physical  
10 size of the capillary determines the density of electric field lines necessary to induce  
11 electrospray.

12 One advantage of electrospray ionization is that the response for an analyte measured  
13 by the mass spectrometer detector is dependent on the concentration of the analyte in the  
14 fluid and independent of the fluid flow rate. The response of an analyte in solution at a given  
15 concentration would be comparable using electrospray ionization combined with mass  
16 spectrometry at a flow rate of 100  $\mu$ L/min compared to a flow rate of 100 nL/min.

17 The process of electrospray ionization at flow rates on the order of nanoliters per  
18 minute has been referred to as "nanoelectrospray". Electrospray into the ion-sampling orifice  
19 of an API mass spectrometer produces a quantitative response from the mass spectrometer  
20 detector due to the analyte molecules present in the liquid flowing from the capillary.

21 Thus, it is desirable to provide an electrospray ionization device for integration  
22 upstream with microchip-based separation devices and for integration downstream with API-  
23 MS instruments.

24 Attempts have been made to manufacture an electrospray device which produces  
25 nanoelectrospray. For example, Wilm and Mann, *Anal. Chem.* 1996, 68, 1-8 describes the  
26 process of electrospray from fused silica capillaries drawn to an inner diameter of 2-4  $\mu$ m at  
flow rates of 20 nL/min. Specifically, a nanoelectrospray at 20 nL/min was achieved from  
a 2  $\mu$ m inner diameter and 5  $\mu$ m outer diameter pulled fused-silica capillary with 600-700  
V at a distance of 1-2 mm from the ion-sampling orifice of an API mass spectrometer.

Ramsey et al., *Anal. Chem.* 1997, 69, 1174-1178 describes nanoelectrospray at 90  
nL/min from the edge of a planar glass microchip with a closed separation channel 10  $\mu$ m  
deep, 60  $\mu$ m wide and 33 mm in length using electroosmotic flow and applying 4.8 kV to the  
fluid exiting the closed separation channel on the edge of the microchip for electrospray  
formation, with the edge of the chip at a distance of 3-5 mm from the ion-sampling orifice

1 of an API mass spectrometer. Approximately 12 nL of the sample fluid collects at the edge  
2 of the chip before the formation of a Taylor cone and stable nanoelectrospray from the edge  
3 of the microchip. However, collection of approximately 12 nL of the sample fluid will result  
4 in remixing of the fluid, thereby undoing the separation done in the separation channel.  
5 Remixing causes band broadening at the edge of the microchip, fundamentally limiting its  
6 applicability for nanoelectrospray-mass spectrometry for analyte detection. Thus,  
7 nanoelectrospray from the edge of this microchip device after capillary electrophoresis or  
8 capillary electrochromatography separation is rendered impractical. Furthermore, because  
9 this device provides a flat surface, and thus a relatively small amount of physical asperity,  
10 for the formation of the electrospray, the device requires an impractically high voltage to  
11 initiate electrospray, due to poor field line concentration.

12 Xue, Q.; Foret, F.; Dunayevskiy, Y. M.; Zavracky, P. M.; McGruer, N.E.; Karger,  
13 B. L. *Anal. Chem.* 1997, 69, 426-430 describes a stable nanoelectrospray from the edge of  
14 a planar glass microchip with a closed channel 25  $\mu\text{m}$  deep, 60  $\mu\text{m}$  wide and 35-50 mm in  
15 length and applying 4.2 kV to the fluid exiting the closed separation channel on the edge of  
16 the microchip for electrospray formation, with the edge of the chip at a distance of 3-8 mm  
17 from the ion-sampling orifice of an API mass spectrometer. A syringe pump is utilized to  
18 deliver the sample fluid to the glass microchip electrosprayer at a flow rate between 100-200  
19 nL/min. The edge of the glass microchip is treated with a hydrophobic coating to alleviate  
20 some of the difficulties associated with nanoelectrospray from a flat surface and which  
21 slightly improves the stability of the nanoelectrospray. Electrospraying in this manner from  
22 a flat surface again results in poor field line concentration and yields an inefficient  
23 electrospray.

24 Desai et al. 1997 *International Conference on Solid-State Sensors and Actuator*,  
25 Chicago, June 16-19, 1997, 927-930 describes a multi-step process to generate a nozzle on  
26 the edge of a silicon microchip 1-3  $\mu\text{m}$  in diameter or width and 40  $\mu\text{m}$  in length and  
applying 4 kV to the entire microchip at a distance of 0.25-0.4 mm from the ion-sampling  
orifice of an API mass spectrometer. This nanoelectrospray nozzle reduces the dead volume  
of the sample fluid. However, the extension of the nozzle from the edge of the microchip  
exposes the nozzle to accidental breakage. Because a relatively high spray voltage was  
utilized and the nozzle was positioned in very close proximity to the mass spectrometer  
sampling orifice, a poor field line concentration and a low efficient electrospray were  
achieved.

In all of the above-described devices, edge-spraying from a monolithic chip is a

poorly controlled process due to the inability to rigorously and repeatably determine the physical form of the chip's edge. In another embodiment of edge-spraying, ejection nozzles, such as small segments of drawn capillaries, are separately and individually attached to the chip's edge. This process is inherently cost-inefficient and unreliable, imposes space constraints in chip design, and is therefore unsuitable for manufacturing.

Thus, it is also desirable to provide an electrospray ionization device with controllable spraying and a method for producing such a device which is easily reproducible and manufacturable in high volumes.

## SUMMARY OF THE INVENTION

The present invention provides a silicon microchip-based electrospray device for producing reproducible, controllable and robust nanoelectrospray ionization of a liquid sample. The electrospray device may be interfaced downstream to an atmospheric pressure ionization mass spectrometer (API-MS) for analysis of the electrosprayed fluid and/or interfaced upstream to a miniaturized liquid phase separation device, which may have, for example, glass, plastic or silicon substrates or wafers.

The electrospray device of the present invention generally comprises a silicon substrate or microchip defining a channel between an entrance orifice on an injection surface and a nozzle on an ejection surface (the major surface) such that the electrospray generated by the electrospray device is generally approximately perpendicular to the ejection surface. The nozzle has an inner and an outer diameter and is defined by an annular portion recessed from the ejection surface. The annular recess extends radially from the outer diameter. The tip of the nozzle is co-planar or level with and does not extend beyond the ejection surface and thus the nozzle is protected against accidental breakage. The nozzle, channel and recessed portion are etched from the silicon substrate by reactive-ion etching and other standard semiconductor processing techniques.

All surfaces of the silicon substrate preferably have a layer of silicon dioxide thereon created by oxidization to electrically isolate the liquid sample from the substrate and the ejection and injection surfaces from each other such that different potential voltages may be individually applied to each surface and the liquid sample. The silicon dioxide layer also provides for biocompatibility. The electrospray apparatus further comprises at least one controlling electrode electrically contacting the substrate through the oxide layer for the application of an electric potential to the substrate.

1 Preferably, the nozzle, channel and recess are etched from the silicon substrate by  
2 reactive-ion etching and other standard semiconductor processing techniques. The injection-  
3 side feature(s), through-substrate fluid channel, ejection-side features, and controlling  
4 electrodes - are formed monolithically from a monocrystalline silicon substrate. That is, they  
5 are formed during the course of and as a result of a fabrication sequence that requires no  
manipulation or assembly of separate components.

6 Because the electrospray device is manufactured using reactive-ion etching and other  
7 standard semiconductor processing techniques, the dimensions of such a device can be very  
8 small, for example, as small as  $2\text{ }\mu\text{m}$  inner diameter and  $5\text{ }\mu\text{m}$  outer diameter. Thus, a nozzle  
9 having, for example,  $5\text{ }\mu\text{m}$  inner diameter and  $250\text{ }\mu\text{m}$  in height only has a volume of  $4.9\text{ pL}$   
10 (picoliter). In contrast, an electrospray device from the flat edge of a glass microchip would  
11 introduce additional dead volume of  $12\text{ nL}$  compared to the volume of a separation channel  
12 of  $19.8\text{ nL}$  thereby allowing remixing of the fluid components and undoing the separation  
done by the separation channel. The micrometer-scale dimensions of the electrospray device  
minimizes the dead volume and thereby increases efficiency and analysis sensitivity.

13 The electrospray device of the present invention provides for the efficient and  
14 effective formation of an electrospray. By providing an electrospray surface from which the  
15 fluid is ejected with dimensions on the order of micrometers, the electrospray device limits  
16 the voltage required to generate a Taylor cone as the voltage is dependent upon the nozzle  
17 diameter, surface tension of the fluid and the distance of the nozzle from the extracting  
18 electrode. The nozzle of the electrospray device provides the physical asperity on the order  
19 of micrometers on which a large electric field is concentrated. Further, the electrospray  
20 device may provide additional electrode(s) on the ejecting surface to which electric  
21 potential(s) may be applied and controlled independent of the electric potentials of the fluid  
22 and the extracting electrode in order to advantageously modify and optimize the electric  
23 field. The combination of the nozzle and the additional electrode(s) thus enhance the electric  
24 field between the nozzle and the extracting electrode. The large electric field, on the order  
25 of  $10^6\text{ V/m}$  or greater and generated by the potential difference between the fluid and  
26 extracting electrode, is thus applied directly to the fluidic cone rather than uniformly  
distributed in space.

The microchip-based electrospray ionization device of the present invention  
provides minimal extra-column dispersion as a result of a reduction in the extra-column  
volume and provides efficient, reproducible, reliable and rugged formation of an  
electrospray. The design of the ionization device is also robust such that the electrospray

1 device can be readily mass-produced in a cost-effective, high-yielding process.

2 In operation, a conductive or partly conductive liquid sample is introduced into the  
3 channel through the entrance orifice on the injection surface. The liquid sample and nozzle  
4 are held at the potential voltage applied to the fluid, either by means of a wire within the fluid  
5 delivery channel to the electrospray device or by means of an electrode formed on the  
6 injection surface isolated from the surrounding surface region and from the substrate. The  
7 electric field strength at the tip of the nozzle is enhanced by the application of a voltage to  
8 the substrate and/or the ejection surface, preferably approximately less than one-half of the  
9 voltage applied to the fluid. Thus, by the independent control of the fluid/nozzle and  
10 substrate/ejection surface voltages, the electrospray device of the present invention allows  
11 the optimization of the electric field lines emanating from the nozzle. Further, when the  
12 electrospray device is interfaced downstream with a mass spectrometry device, the  
13 independent control of the fluid/nozzle and substrate/ejection surface voltages also allows  
14 for the direction and optimization of the electrospray into an acceptance region of the mass  
15 spectrometry device.

16 The electrospray device of the present invention may be placed 1-2 mm or up to 10  
17 mm from the orifice of an API mass spectrometer to establish a stable nanoelectrospray at  
18 flow rates as low as 20 nL/min with a voltage of, for example, 700 V applied to the nozzle  
19 and 0-350 V applied to the substrate and/or the planar ejection surface of the silicon  
20 microchip.

21 An array or matrix of multiple electrospray devices of the present invention may be  
22 manufactured on a single microchip as silicon fabrication using standard, well-controlled  
23 thin-film processes not only eliminates handling of such micro components but also allows  
24 for rapid parallel processing of functionally alike elements. The nozzles may be radially  
25 positioned about a circle having a relatively small diameter near the center of the chip. Thus,  
26 the electrospray device of the present invention provides significant advantages of time and  
cost efficiency, control, and reproducibility. The low cost of these electrospray devices  
allows for one-time use such that cross-contamination from different liquid samples may be  
eliminated.

The electrospray device of the present invention can be integrated upstream with  
miniaturized liquid sample handling devices and integrated downstream with an API mass  
spectrometer. The electrospray device may be chip-to-chip or wafer-to-wafer bonded to  
silicon microchip-based liquid separation devices capable of, for example, capillary  
electrophoresis, capillary electrochromatography, affinity chromatography, liquid



1 a common point for subsequent sequential analysis. The multiple nozzles of the electrospray  
2 devices may be radially positioned about a circle having a relatively small diameter near the  
center of the single chip.

3 The radially distributed array of electrospray nozzles on a multi-system chip may be  
4 interfaced with a sampling orifice of a mass spectrometer by positioning the nozzles near the  
5 sample orifice. The tight radial configuration of the electrospray nozzles allows the  
positioning thereof in close proximity to the sampling orifice of a mass spectrometer.

6 The multi-system chip thus provides a rapid sequential chemical analysis system  
7 fabricated using microelectromechanical systems (MEMS) technology. For example, the  
8 multi-system chip enables automated, sequential separation and injection of a multiplicity  
9 of samples, resulting in significantly greater analysis throughput and utilization of the mass  
10 spectrometer instrument for, for example, high-throughput detection of compounds for drug  
discovery.

#### 11 BRIEF DESCRIPTION OF THE DRAWINGS

12 The file of this patent contains at least one drawing executed in color. Copies of this  
13 patent with color drawings will be provided by the Patent and Trademark Office upon request  
14 and payment of the necessary fee.

15 FIG. 1 shows a schematic of an electrospray system;

16 FIG. 2 shows a perspective view of an electrospray device of the present invention;

17 FIG. 3 shows a plan view of the electrospray device of FIG. 2;

18 FIG. 4 shows a cross-sectional view of the electrospray device of FIG. 3 taken along  
line 4-4;

19 FIG. 5 shows a schematic of an electrospray system comprising an electrospray  
device of the present invention;

20 FIG. 6 shows a plan view of an electrospray device having multiple electrodes on  
the ejection surface of the device;

21 FIG. 7 shows a cross-sectional view of the electrospray device of FIG. 6 taken along  
22 line 7-7;

23 FIG. 8 illustrates a feedback control circuit incorporating an electrospray device of  
the present invention;

24 FIGS. 9-20G show an example of a fabrication sequence of the electrospray device;



1 FIG. 21 A shows a cross-sectional view of a piezoelectric pipette positioned at a  
2 distance from and for delivery of a fluid sample to the entrance orifice of the electrospray  
device;

3 FIG. 21 B shows a cross-sectional view of a capillary for delivery of a fluid sample  
4 to and prior to attachment to the entrance orifice of the electrospray device;

5 FIG. 22 shows a schematic of a single integrated system comprising an upstream  
6 fluid delivery device and an electrospray device having a homogeneous interface with the  
fluid delivery device;

7 FIG. 23A shows an exploded perspective view of a chip-based combinatorial  
8 chemistry system comprising a reaction well block and a daughter plate;

9 FIG. 23B shows a cross-sectional view of the chip-based combinatorial chemistry  
system of FIG. 23A taken along line 23B-23B;

10 FIGS. 24A and 24B shows a real Taylor cone emanating from an integrated silicon  
11 chip-based nozzle;

12 FIGS. 24C and 24D are perspective and side cross-sectional views, respectively, of  
the electrospray device and mass spectrometry system of FIGS. 24A and 24B;

13 FIG. 24E shows a mass spectrum of 1  $\mu\text{g/mL}$  PPG425 in 50% water, 50% methanol  
14 containing 0.1% formic acid, 0.1% acetonitrile and 2 mM ammonium acetate, collected at  
a flow rate of 333 nL/min;

15 FIG. 25A shows an exploded perspective view of a liquid chromatography device  
16 for homogeneous integration with the electrospray device of the present invention;

17 FIG. 25B shows a cross-sectional view of the liquid chromatography device of FIG.  
25A taken along line 25B-25B;

18 FIG. 26 shows a plan view of a liquid chromatography device having an exit orifice  
19 forming an off-chip interconnection with an off-chip device;

20 FIG. 27 shows a plan view of a liquid chromatography device having an exit orifice  
forming an on-chip interconnection with another on-chip device;

21 FIGS. 28-29 show cross-sectional views of liquid chromatography devices having  
22 alternative configurations;

23 FIGS. 30-35 show plan views of liquid chromatography devices having alternative  
configurations;

24 FIGS. 36A-46C show an example of a fabrication sequence of the liquid  
25 chromatography device;

26

1 FIG. 47 shows a cross-sectional view of a system comprising a liquid  
2 chromatography device homogenously integrated with an electrospray device;

3 FIG. 48 shows a plan view of the system of FIG. 47; and

4 FIG. 49 shows a detailed view of the nozzles of the system of FIG. 47.

### 5 DETAILED DESCRIPTION OF THE INVENTION

6 An aspect of the present invention provides a silicon microchip-based electrospray  
7 device for producing electrospray ionization of a liquid sample. The electrospray device may  
8 be interfaced downstream to an atmospheric pressure ionization mass spectrometer (API-  
9 MS) for analysis of the electrosprayed fluid. Another aspect of the invention is an integrated  
10 miniaturized liquid phase separation device, which may have, for example, glass, plastic or  
11 silicon substrates integral with the electrospray device. The descriptions that follow present  
12 the invention in the context of a liquid chromatograph separation device. However, it will  
13 be readily recognized that equivalent devices can be made that utilize other microchip-based  
14 separation devices. The following description is presented to enable any person skilled in the  
15 art to make and use the invention. Descriptions of specific applications are provided only as  
16 examples. Various modifications to the preferred embodiment will be readily apparent to  
17 those skilled in the art, and the general principles defined herein may be applied to other  
18 embodiments and applications without departing from the spirit and scope of the invention.  
19 Thus, the present invention is not intended to be limited to the embodiments shown, but is  
20 to be accorded the widest scope consistent with the principles and features disclosed herein.

### 21 **ELECTROSPRAY DEVICE**

22 FIGS. 2-4 show, respectively, a perspective view, a plan view and a crosssectional  
23 view of an electrospray device **100** of the present invention. The electrospray apparatus of  
24 the present invention generally comprises a silicon substrate or microchip or wafer **102**  
25 defining a channel **104** through substrate **102** between an entrance orifice **106** on an injection  
26 surface **108** and a nozzle **110** on an ejection surface **112**. The channel may have any suitable  
cross-sectional shape such as circular or rectangular. The nozzle **110** has an inner and an  
outer diameter and is defined by a recessed region **114**. The region **114** is recessed from the  
ejection surface **112**, extends outwardly from the nozzle **110** and may be annular. The tip  
of the nozzle **110** does not extend beyond and is preferably coplanar or level with the ejection  
surface **112** to thereby protect the nozzle **110** from accidental breakage.

Preferably, the injection surface **108** is opposite the ejection surface **112**. However,  
although not shown, the injection surface may be adjacent to the ejection surface such that

1 the channel extending between the entrance orifice and the nozzle makes a turn within the  
2 device. In such a configuration, the electrospray device would comprise two substrates  
3 bonded together. The first substrate may define a through-substrate channel extending  
4 between a bonding surface and the ejection surface, opposite the bonding surface. The first  
5 substrate may further define an open channel recessed from the bonding surface extending  
6 from an orifice of the through-substrate channel and the injection surface such that the  
7 bonding surface of the second substrate encloses the open channel upon bonding of the first  
8 and second substrates. Alternatively, the second substrate may define an open channel  
9 recessed from the bonding surface such that the bonding surface of the first substrate  
10 encloses the open channel upon bonding of the first and second substrates. In yet another  
11 variation, the first substrate may further define a second through-substrate channel while the  
12 open channel extends between the two through-substrate channels. Thus, the injection  
13 surface is the same surface as the ejection surface.

14 A grid-plane region **116** of the ejection surface **112** is exterior to the nozzle **110** and  
15 to the recessed region **114** and may provide a surface on which a layer of conductive material  
16 **119**, including a conductive electrode **120**, may be formed for the application of an electric  
17 potential to the substrate **102** to modify the electric field pattern between the ejection surface  
18 **112**, including the nozzle tip **110**, and the extracting electrode **54**. Alternatively, the  
19 conductive electrode may be provided on the injection surface **108** (not shown).

20 The electrospray device **100** further comprises a layer of silicon dioxide **118** over  
21 the surfaces of the substrate **102** through which the electrode **120** is in contact with the  
22 substrate **102** either on the ejection surface **112** or on the injection surface **108**. The silicon  
23 dioxide **118** formed on the walls of the channel **104** electrically isolates a fluid therein from  
24 the silicon substrate **102** and thus allows for the independent application and sustenance of  
25 different electrical potentials to the fluid in the channel **104** and to the silicon substrate **102**.  
26 The ability to independently vary the fluid and substrate potentials allows the optimization  
of the electrospray through modification of the electric field line pattern, as described below.  
Alternatively, the substrate **102** can be controlled to the same electrical potential as the fluid  
when appropriate for a given application.

As shown in FIG. 5, to generate an electrospray, fluid may be delivered to the  
entrance orifice **106** of the electrospray device **100** by, for example, a capillary **52** or  
micropipette. The fluid is subjected to a potential voltage  $V_{\text{fluid}}$  via a wire (not shown)  
positioned in the capillary **52** or in the channel **104** or via an electrode (not shown) provided  
on the injection surface **108** and isolated from the surrounding surface region and the

1 substrate **102** . A potential voltage  $V_{\text{substrate}}$  may also be applied to the electrode **120** on the  
2 grid-plane **116**, the magnitude of which is preferably adjustable for optimization of the  
3 electrospray characteristics. The fluid flows through the channel **104** and exits or is ejected  
4 from the nozzle **110** in the form of very fine, highly charged fluidic droplets **58**. The  
5 electrode **54** may be held at a potential voltage  $V_{\text{extract}}$  such that the electrospray is drawn  
6 toward the extracting electrode **54** under the influence of an electric field. As it is the  
7 relative electric potentials which affect the electric field, the potential voltages of the fluid,  
8 the substrate and the extracting electrode may be easily adjusted and modified to achieve the  
9 desired electric field. Generally, the magnitude of the electric field should not exceed the  
10 dielectric breakdown strength of the surrounding medium, typically air.

11 In one embodiment, the nozzle **110** may be placed up to 10 mm from the sampling  
12 orifice of an API mass spectrometer serving as the extracting electrode **54**. A potential  
13 voltage  $V_{\text{fluid}}$  ranging from approximately 500-1000 V, such as 700 V, is applied to the fluid.  
14 The potential voltage of the fluid  $V_{\text{fluid}}$  may be up to 500 V/ $\mu\text{m}$  of silicon dioxide on the  
15 surface of the substrate **102** and may depend on the surface tension of the fluid being sprayed  
16 and the geometry of the nozzle **110**. A potential voltage of the substrate  $V_{\text{substrate}}$  of  
17 approximately less than half of the fluid potential voltage  $V_{\text{fluid}}$ , or 0-350 V, is applied to the  
18 electrode on the grid-plane **116** to enhance the electric field strength at the tip of the nozzle  
19 **110**. The extracting electrode **54** may be held at or near ground potential  $V_{\text{extract}}$  (0 V). Thus,  
20 a nanoelectrospray of a fluid introduced to the electrospray device **100** at flow rates less than  
21 1,000 nL/min is drawn toward the extracting electrode **54** under the influence of the electric  
22 field.

23 The nozzle **110** provides the physical asperity for concentrating the electric field  
24 lines emanating from the nozzle **110** in order to achieve efficient electrospray. The nozzle  
25 **110** also forms a continuation of and serves as an exit orifice of the through-substrate channel  
26 **104**. Furthermore, the recessed region **114** serves to physically isolate the nozzle **110** from  
the grid-plane region **116** of the ejection surface **112** to thereby promote the concentration  
of electric field lines and to provide electrical isolation between the nozzle **110** and the grid-  
plane region **116**. The present invention allows the optimization of the electric field lines  
emanating from the nozzle **110** through independent control of the potential voltage  $V_{\text{fluid}}$   
of the fluid and nozzle **110** and the potential voltage  $V_{\text{substrate}}$  of the electrode on the grid-plane  
**116** of the ejection surface **112**.

In addition to the electrode **120**, one or more additional conductive electrodes may  
be provided on the silicon dioxide layer **118** on the ejection surface **112** of the substrate **102**.

FIGS. 6 and 7 show, respectively, a plan view and a cross-sectional view of an example of an electrospray device **100'** wherein the conductive layer **119** defines three additional electrodes **122, 124, 126** on the ejection surface **112** of the substrate **102**. Because the silicon dioxide layer **118** on the ejection surface **112** electrically isolates the silicon substrate **102** from the additional electrodes **122, 124, 126** on the ejection surface **112** and because the additional electrodes **122, 124, 126** are physically separated from each other, the electrical potential applied to each of the additional electrodes **122, 124, 126** can be controlled independently from each other, from the substrate **102** and from the fluid. Thus, additional electrodes **122, 124, 126** may be utilized to further modify the electric field line pattern to effect, for example, a steering and/or shaping of the electrospray. Although shown to be of similar sizes and shapes, electrode **120** and additional electrodes **122, 124, 126** may be of any same or different suitable shapes and sizes.

To further control and optimize the electrospray, a feedback control circuit **130** as shown in FIG. 8 may also be provided with the electrospray device **100**. The feedback circuit **130** includes an optimal spray attribute set point **132**, a comparator and voltage control **134** and one or more spray attribute sensors **136**. The optimal spray attribute set point **132** is set by an operator or at a determined or default value. The one or more spray attribute sensors **136** detect one or more desired attributes of the electrospray from the electrospray device **100**, such as the electrospray ion current and/or the spatial concentration of the spray pattern. The spray attribute sensor **136** sends signals indicating the value of the desired attribute of the electrospray to the comparator and voltage control **134** which compares the indicated value of the desired attribute with the optimal spray attribute set point **132**. The comparator and voltage control **134** then applies potential voltages  $V_{\text{fluid}}$ ,  $V_{\text{substrate}}$  to the fluid and the silicon substrate **102**, respectively, which may be independently varied to optimize the desired electrospray attribute. Although not shown, the comparator and voltage control **134** may apply independently controlled additional potential voltages to each of one or more additional conductive electrodes.

The feedback circuit **130** may be interfaced with the electrospray device **100** in any suitable fashion. For example, the feedback circuit **130** may be fabricated as an integrated circuit on the electrospray device **100**, as a separate integrated circuit with electrical connection to the electrospray device **100**, or as discrete components residing on a common substrate electrically connected to the substrate of the electrospray device.

Dimensions of the electrospray device **100** can be determined according to various factors such as the specific application, the layout design as well as the upstream and/or

In one currently preferred embodiment, the silicon substrate **102** of the electrospray device **100** is approximately 250-600  $\mu\text{m}$  in thickness and the cross-sectional area of the channel **104** is less than approximately 50,000  $\mu\text{m}^2$ . Where the channel **104** has a circular cross-sectional shape, the channel **104** and the nozzle **110** have an inner diameter of up to 250  $\mu\text{m}$ , more preferably up to 145  $\mu\text{m}$ ; the nozzle **110** has an outer diameter of up to 255  $\mu\text{m}$ , more preferably up to 150  $\mu\text{m}$ ; and nozzle **110** has a height of (and the recessed portion **114** has a depth of) up to 500  $\mu\text{m}$ . The recessed portion **114** preferably extends up to 1000  $\mu\text{m}$  outwardly from the nozzle **110**. The silicon dioxide layer **118** has a thickness of approximately 1-4  $\mu\text{m}$ , preferably 1-2  $\mu\text{m}$ .

The fabrication of the electrospray device **100** will now be explained with reference to FIGS. 9-20B. The electrospray device **100** is preferably fabricated as a monolithic silicon integrated circuit utilizing established, well-controlled thin-film silicon processing techniques such as thermal oxidation, photolithography, reactivation etching (RIE), ion implantation, and metal deposition. Fabrication using such silicon processing techniques facilitates massively parallel processing of similar devices, is time- and cost-efficient, allows for tighter control of critical dimensions, is easily reproducible, and results in a wholly integral device, thereby eliminating any assembly requirements. Further, the fabrication sequence may be easily extended to create physical aspects or features on the injection surface and/or ejection surface of the electrospray device to facilitate interfacing and connection to a fluid delivery system or to facilitate integration with a fluid delivery sub-system to create a single integrated system.

FIGS. 9A-11 illustrate the processing steps for the injection side of the substrate in fabricating the electrospray device **100** of the present invention. Referring to the plan and cross-sectional views, respectively, of FIGS. 9A and 9B, a double-side polished silicon wafer substrate **200** is subjected to an elevated temperature in an oxidizing ambient to grow a layer or film of silicon dioxide **202** on the injection side **203** and a layer or film of silicon dioxide **204** on the ejection side **205** of the substrate **200**. Each of the resulting silicon dioxide layers

1 **202, 204** has a thickness of approximately 1-2  $\mu\text{m}$ . The silicon dioxide layers **202, 204**  
2 provide electrical isolation and also serve as masks for subsequent selective etching of  
3 certain areas of the silicon substrate **200**.

4 A film of positive-working photoresist **206** is deposited on the silicon dioxide layer  
5 **202** on the injection side **203** of the substrate **200**. An area of the photoresist **206**  
6 corresponding to the entrance to a through-wafer channel which will be subsequently etched  
7 is selectively exposed through a mask by an optical lithographic exposure tool passing short-  
8 wavelength light such as blue or near-ultraviolet at wavelengths of 365, 405, or 436  
9 nanometers.

10 As shown in the plan and cross-sectional views, respectively, of FIGS. 10A and 10B,  
11 after development of the photoresist **206**, the exposed area **208** of the photoresist is removed  
12 and open to the underlying silicon dioxide layer **202** while the unexposed areas remain  
13 protected by photoresist **206'**. The exposed area **210** of the silicon dioxide layer **202** is then  
14 etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to the  
15 protective photoresist **206'** until the silicon substrate **200** is reached. The remaining  
16 photoresist is removed in an oxygen plasma or in an actively oxidizing chemical bath like  
17 sulfuric acid ( $\text{H}_2\text{SO}_4$ ) activated with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).

18 As shown in the cross-sectional view of FIG. 11, an injection side portion **212** of the  
19 through channel in the silicon substrate **200** is vertically etched by another fluorine-based  
20 etch. An advantage of the fabrication process described herein is that the dimensions of the  
21 through channel, such as the aspect ratio (depth to width), can be reliably and reproducibly  
22 limited and controlled. In the case where the etch aspect ratio of the processing equipment  
23 is a limiting factor, it is possible to overcome this limitation by a first etch on one side of a  
24 wafer followed by a second etch on a second side of the wafer. For example, a current  
25 silicon etch process is generally limited to an etch aspect ratio of 30:1, such that a channel  
26 having a diameter less than approximately 10  $\mu\text{m}$  through a substrate **200** having customary  
thickness approximately 250-600  $\mu\text{m}$  would be etched from both surfaces of the substrate  
**200**.

The depth of the channel portion **212** should be at or above a minimum in order to  
connect with another portion of the through channel etched from the ejection side **205** of the  
substrate **200**. The desired depth of the recessed region **114** on the ejection side **205**  
determines approximately how far the ejection side portion **220** of the channel **104** is etched.  
The remainder of the channel **104**, the injection side portion **212**, is etched from the injection  
side. The minimum depth of channel portion **212** is typically 50  $\mu\text{m}$ , although the exact etch

1 depth above the minimum etch depth does not impact the device performance or yield of the  
2 electropray device.

3 **Ejection surface processing: nozzle and surrounding surface structure**

4 FIGS. 12-20B illustrate the processing steps for the ejection side **205** of the substrate  
5 **200** in fabricating the electropray device **100** of the present invention. As shown in the  
6 cross-sectional view in FIG. 12, a film of positive-working photoresist **214** is deposited on  
7 the silicon dioxide layer **204** on the ejection side **205** of the substrate **200**. Patterns on the  
8 ejection side **205** are aligned to those previously formed on the injection side **203** of the  
9 substrate **200**. Because silicon and its oxide are inherently relatively transparent to light in  
10 the infrared wavelength range of the spectrum, i.e. approximately 70-1000 nanometers, the  
11 extant pattern on the injection side **203** can be distinguished with sufficient clarity by  
12 illuminating the substrate **200** from the patterned injection side **203** with infrared light. Thus,  
13 the mask for the ejection side **205** can be aligned within required tolerances.

14 After alignment, certain areas of the photoresist **214** corresponding to the nozzle and  
15 the recessed region are selectively exposed through an ejection side mask by an optical  
16 lithographic exposure tool passing short-wavelength light, such as blue or near-ultraviolet  
17 at wavelengths of 365, 405, or 436 nanometers. As shown in the plan and cross-sectional  
18 views, respectively, of FIGS. 13A and 13B, the photoresist **214** is then developed to remove  
19 the exposed areas of the photo resist such that the nozzle area **216** and recessed region area  
20 **218** are open to the underlying silicon dioxide layer **204** while the unexposed areas remain  
21 protected by photoresist **214'**. The exposed areas **216**, **218** of the silicon dioxide layer **204**  
22 are then etched by a fluorine-based plasma with a high degree of anisotropy and selectivity  
23 to the protective photoresist **214'** until the silicon substrate **200** is reached.

24 As shown in the cross-sectional view of FIG. 14, the remaining photoresist **214'**  
25 provides additional masking during a subsequent fluorine based silicon etch to vertically etch  
26 certain patterns into the ejection side **205** of the silicon substrate **200**. The remaining  
photoresist **214'** is then removed in an oxygen plasma or in an actively oxidizing chemical  
bath like sulfuric acid ( $H_2SO_4$ ) activated with hydrogen peroxide ( $H_2O_2$ ).

The fluorine-based etch creates a channel **104** through the silicon substrate **200** by  
forming an ejection side portion **220** of the channel **104**. The fluorine based etch also creates  
an ejection nozzle **110**, a recessed region **114** exterior to the nozzle **110** and a grid-plane  
region **116** exterior to the nozzle **110** and to the recessed region **114**. The grid-plane region  
**116** is preferably co-planar with the tip of the nozzle **110** so as to physically protect the  
nozzle **110** from casual abrasion, stress fracture in handling and/or accidental breakage. The



The fabrication sequence confers superior mechanical stability to the fabricated electrospray device by etching the features of the electrospray device from a monocrystalline silicon substrate without any need for assembly. The fabrication sequence allows for the control of the nozzle height by adjusting the relative amounts of injection side and ejection side silicon etching. Further, the lateral extent and shape of the recessed region **114** can be controlled independently of its depth, which affects the nozzle height and which is determined by the extent of the etch on the ejection side of the substrate. Control of the lateral extent and shape of the recessed region **114** provides the ability to modify and control the electric field pattern between the electrospray device **100** and an extracting electrode.

## Oxidation for electrical isolation

As shown in the cross-sectional view of FIG. 15, a layer of silicon dioxide **221** is grown on all silicon surfaces of the substrate **200** by subjecting the silicon substrate **200** to elevated temperature in an oxidizing ambient. For example, the oxidizing ambient may be an ultra-pure steam produced by oxidation of hydrogen for a silicon dioxide thickness greater than approximately several hundred nanometers or pure oxygen for a silicon dioxide thickness of approximately several hundred nanometers or less. The layer of silicon dioxide **221** over all silicon surfaces of the substrate **200** electrically isolates a fluid in the channel from the silicon substrate **200** and permits the application and sustenance of different electrical potentials to the fluid in the channel **104** and to the silicon substrate **200**.

All silicon surfaces are oxidized to form silicon dioxide with a thickness that is controllable through choice of temperature and time of oxidation. The final thickness of the silicon dioxide can be selected to provide the desired degree of electrical isolation in the device, where a thicker layer of silicon dioxide provides a greater resistance to electrical breakdown.

### Metallization for electric field control

FIGS. 16-20B illustrate the formation of a single conductive electrode electrically connected to the substrate **200** on the ejection side **205** of the substrate **200**. As shown in the cross-sectional view of FIG. 16, a film of positive-working photoresist **222** is deposited over the silicon dioxide layer on the ejection side **205** of the substrate **200**. An area of the photoresist **222** corresponding to the electrical contact area between the electrode and the substrate **200** is selectively exposed through another mask by an optical lithographic

1 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths  
2 of 365, 405, or 436 nanometers.

3 The photoresist **222** is then developed to remove the exposed area **224** of the  
4 photoresist such that the electrical contact area between the electrode and the substrate **200**  
5 is open to the underlying silicon dioxide layer **204** while the unexposed areas remain  
6 protected by photoresist **222'**. The exposed area **224** of the silicon dioxide layer **204** is then  
7 etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to the  
8 protective photoresist **222'** until the silicon substrate **200** is reached, as shown in the cross-  
9 sectional view of FIG. 17.

10 Referring now to the cross-sectional view of FIG. 18, the remaining photoresist is  
11 then removed in an oxygen plasma or in an actively oxidizing chemical bath like sulfuric  
12 acid ( $\text{H}_2\text{SO}_4$ ) activated with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Utilizing the patterned ejection side  
13 silicon dioxide layer **204** as a mask, a high-dose implantation is made to form an implanted  
14 region **225** to ensure a low-resistance electrical connection between the electrode and the  
15 substrate **200**. A conductive film **226** such as aluminum may be uniformly deposited on the  
16 ejection side **205** of the substrate **200** by thermal or ejection-beam evaporation to form an  
17 electrode **120**. The thickness of the conductive film **226** is preferably approximately 3000  
18 Å, although shown having a larger thickness for clarity.

19 The conductive film **226** may be created by any method which does not produce a  
20 continuous film of the conductive material on the side walls of the ejection nozzle **110**. Such  
21 a continuous film would electrically connect the fluid in the channel **104** and the substrate  
22 **200** so as to prevent the independent control of their respective electrical potentials. For  
23 example, the conductive film may be deposited by thermal or electron-beam evaporation of  
24 the conductive material, resulting in line-of-sight deposition on presented surfaces. Orienting  
25 the substrate **200** such that the side walls of the ejection nozzle **110** are out of the line-of-  
26 sight of the evaporation source ensures that no conductive material is deposited as a  
continuous film on the side walls of the ejection nozzle **110**. Sputtering of conductive  
material in a plasma is an example of a deposition technique which would result in  
deposition of conductive material on all surfaces and thus is undesirable.

One or more additional conductive electrodes may be easily formed on the ejection  
side **205** of the substrate **200**, as described above with reference to FIGS. 6 and 7. As shown  
in the cross-sectional view of FIG. 19, a film of positive-working photoresist **228** is  
deposited over the conductive film **226** on the ejection side **205** of the substrate **200**. Certain  
areas of the photoresist **228** corresponding to the physical spaces between the electrodes are

1 selectively exposed through another mask by an optical lithographic exposure tool passing  
2 short-wavelength light, such as blue or near-ultraviolet at wavelengths of 365, 405, or 436  
nanometers.

3 Referring now to the plan and cross-sectional views of FIGS. 20A and 20B, the  
4 photoresist **228** is developed to remove the exposed areas **230** of the photoresist such that the  
5 exposed areas are open to the underlying conductive film **226** while the unexposed areas  
6 remain protected by photoresist **228'**. The exposed areas **230** of the conductive film **226** are  
7 then etched using either a wet chemical etch or a reactive-ion etch, as appropriate for the  
8 particular conductive material. The etch is either selective to the underlying silicon dioxide  
layer **204** or the etch must be terminated on the basis of etch rate and time of etch. Finally,  
the remaining photoresist is then removed in an oxygen plasma.

9 The etching of the conductive film **226** to the underlying silicon dioxide layer **204**  
10 results in physically and electrically separate islands of conductive material or electrodes.  
11 As described above, these electrodes can be controlled independently from the silicon  
12 substrate or channel fluid because they are electrically isolated from the substrate by the  
13 silicon dioxide and from each other by physical separation. They can be used to further  
14 modify the electric field line pattern and thereby effect a steering and/or shaping of the  
electrosprayed fluid. This step completes the processing and fabrication sequence for the  
electrospray device **100**.

15 As described above, the conductive electrode for application of an electrical  
16 potential to the substrate of the electrospray device may be provided on the injection surface  
17 rather than the ejection surface. The fabrication sequence is similar to that for the conductive  
18 electrode provided on the ejection side **205** of the substrate **200**. FIGS. 20C-20G illustrate  
19 the formation of a single conductive electrode electrically connected to the substrate **200** on  
the injection side **203** of the substrate **200**.

20 As shown in the cross-sectional view of FIG. 20C, a film of positive-working  
21 photoresist **232** is deposited over the silicon dioxide layer on the injection side **203** of the  
22 substrate **200**. An area of the photoresist **232** corresponding to the electrical contact area  
23 between the electrode and the substrate **200** is selectively exposed through another mask by  
an optical lithographic exposure tool passing shortwavelength light, such as blue or near-  
ultraviolet at wavelengths of 365, 405, or 436 nanometers.

24 The photoresist **232** is then developed to remove the exposed area **234** of the  
25 photoresist such that the electrical contact area between the electrode and the substrate **200**  
26 is open to the underlying Silicon dioxide layer **202** while the unexposed areas remain

As shown in the cross-sectional view of FIG. 20F, another film of positive-working photoresist **240** is deposited over the conductive film **238** on the injection side **203** of the substrate **200**. An area of the photoresist **240** corresponding to the region adjacent to the

1 entrance orifice **106** on the injection side **203** is selectively exposed through another mask  
2 by an optical lithographic exposure tool passing short-wavelength light, such as blue or near-  
ultraviolet at wavelengths of 365, 405, or 436 nanometers.

3 The photoresist **240** is then developed to remove the exposed area **242** of the  
4 photoresist such that the region adjacent to the entrance orifice **106** on the injection side **203**  
5 is open to the underlying conductive film **238** while the unexposed areas remain protected  
6 by photoresist **240'**. The exposed area **242** of the conductive film **238** is then etched by, for  
7 example, a chlorine-based plasma with a high degree of anisotropy and selectivity to the  
8 protective photoresist **240'** until the silicon dioxide layer **203** is reached, as shown in the  
cross-sectional view of FIG. 20G.

9 The specific technique for etching the conductive film **238** may be determined by  
10 the specific conductive material deposited. For example, aluminum may be etched either in  
11 a wet chemical bath using standard aluminum etchant or in a plasma using reactive-ion  
12 etching (RIE) and chlorine-based gas chemistry. Utilization of standard wet aluminum  
13 etchant to etch an aluminum film may be preferred as such wet etching may facilitate the  
14 removal of any undesired conductive material deposited in the channel **104** via the entrance  
orifice **106**. Further, although chlorine-based reactive-ion etching may be utilized, such  
etching may lead to aluminum corrosion if removal of the photoresist is delayed.

15 Forming the electrode on the injection surface for application of an electric potential  
16 to the substrate of the electrospray device may provide several advantages. For example,  
17 because the ability to uniformly coat photoresist on a surface is limited by nonplanar surface  
18 topology, coating photoresist on the much flatter injection side results in a more uniform and  
19 continuous photoresist film than coating photoresist on the ejection side. The uniformity and  
20 continuity of the photoresist film directly and positively impact the reliability and yield, at  
least in part because failure of photoresist coverage would allow subsequent etching of  
silicon dioxide in undesired locations during the etching of exposed areas **224**, **234**.

21 Another advantage of forming the electrode on the injection surface is the greater  
22 flexibility and reliability in the conductive material deposition step because the interior  
23 surfaces of the nozzle are not coated by the conductive material deposited onto the injection  
24 surface rather than onto the ejection surface of the electrospray device. As a result,  
25 sputtering may be utilized as a deposition technique to ensure conformal coating of the  
26 conductive material and electrical continuity from the surface to the substrate contact.  
Further, the provision of the electrode on the injection surface does not preclude the  
deposition and patterning of additional conductive electrodes on the ejection side to further

1 modify the electric field line pattern to effect, for example, a steering and/or shaping of the  
2 electropray, as such additional electrodes do not required electrical contact to the substrate.

3 The ability to form the electrode on the injection surface may also be advantageous  
4 in certain applications where physical constraints, such as in packaging, may dictate the need  
for injection-side rather than ejection-side electrical connection.

5 The above described fabrication sequence for the electropray device **100** can be  
6 easily adapted to and is applicable for the simultaneous fabrication of a single monolithic  
7 system comprising multiple electropray devices including multiple channels and/or multiple  
8 ejection nozzles embodied in a single monolithic substrate. Further, the processing steps  
9 may be modified to fabricate similar or different electropray devices merely by, for example,  
modifying the layout design and/or by changing the polarity of the photomask and utilizing  
negative-working photoresist rather than utilizing positive-working photoresist.

10 Further, although the fabrication sequence is described in terms of fabricating a  
11 single electropray device, the fabrication sequence facilitates and allows for massively  
12 parallel processing of similar devices. The multiple electropray devices or systems  
13 fabricated by massively parallel processing on a single wafer may then be cut or otherwise  
separated into multiple devices or systems.

## 14 **INTERFACE OR INTEGRATION OF THE ELECTROSPRAY DEVICE**

### 15 **Downstream Interface or Integration of the Electropray Device**

16 The electropray device **100** may be interfaced or integrated downstream to a  
17 sampling device, depending on the particular application. For example, the analyte may be  
18 electrosprayed onto a surface to coat that surface or into another device for purposes of  
conveyance, analysis, and/or synthesis. As described above with reference to FIG. 5, highly  
charged droplets are formed at atmospheric pressure by the electropray device **100** from  
nanoliter-scale volumes of an analyte. The highly charged droplets produce gas-phase ions  
upon sufficient evaporation of solvent molecules which may be sampled, for example,  
through an orifice of an atmospheric pressure ionization mass spectrometer (API-MS) for  
analysis of the electrosprayed fluid.

### 22 **Upstream Interface or Integration of the Electropray Device**

23 Referring now to FIGS. 21-23, fluid may be delivered to the entrance orifice of the  
24 electropray device in any suitable manner by upstream interface or integration with one or  
25 more fluid delivery devices, such as piezoelectric pipettes, micropipettes, capillaries and  
26 other types of microdevices. The fluid delivery device may be a separate component to form  
a heterogeneous interface with the entrance orifice of the electropray device. Alternatively,

1 the fluid delivery device may be integrated with the electrospray device to form a  
2 homogeneous interface with the entrance orifice of the electrospray device.

3 FIGS. 21A and 21B illustrate examples of fluid delivery devices forming  
4 heterogeneous interfaces with the entrance orifice of the electrospray device. Preferably, the  
5 heterogeneous interface is a non-contacting interface where the fluid delivery device  
6 and the electrospray device are physically separated and do not contact. For example, as  
7 shown in the cross-sectional view of FIG. 21A, a piezoelectric pipette 300 is positioned at  
8 a distance above the injection surface 108 of the electrospray device 100A. The piezoelectric  
9 pipette 300 deposits a flow of microdroplets, each approximately 200 pL in volume, into the  
10 channel 104 through the entrance orifice 106A. Preferably, the electrospray device 100A  
11 provides an entrance well 302 at the entrance orifice 106A for containing the sample fluid  
12 prior to entering the channel 104 particularly when it is desirable to spray a volume of fluid  
13 greater than the volume of the through-substrate channel 104 and continual supply of fluid  
14 is not feasible such as when using the piezoelectric pipette 300. The entrance well 302  
15 preferably has a volume of 0.1 nL to 100 nL. Furthermore, to apply an electric potential to  
16 the fluid, an entrance well electrode 304 may be provided on a surface of the entrance well  
17 302 parallel to the injection surface 108. Alternatively, a wire (not shown) may be  
18 positioned in channel 104 via the entrance orifice 106A. Preferably, some fluid is present  
19 in the entrance well 302 to ensure electrical contact between the fluid and the entrance well  
20 electrode 304.

21 Alternatively, the heterogeneous interface may be a contacting interface where a  
22 fluid delivery device is attached by any suitable method, such as by epoxy bonding, to the  
23 electrospray device to form a continuous sealed flow path between the upstream fluid source  
24 and the channel of the electrospray device. For example, FIG. 21B shows a cross-sectional  
25 view of a capillary 306 prior to attachment to the entrance orifice 106 of the electrospray  
26 device 100B. The injection surface 108 of the electrospray device 100B may be adapted to  
facilitate attachment of the capillary 306. Such features can be easily designed into the mask  
for the injection side of the substrate and can be simultaneously formed with the injection  
side portion of the channel during the etching performed on the injection-side.

For example, where the inner diameter of the capillary 306 is greater than that of the  
channel 104 and the entrance orifice 106, the electrospray device 100B preferably defines a  
region 308 recessed from the injection surface 108 to form a mating collar for mating and  
affixing with the capillary 306. Thus, capillary 306 may be positioned and attached in the  
recessed region 308 such that the exit orifice 310 portion of the capillary 302 is positioned

1 around the entrance orifice **106**. Further, the electrospray device **100B** may optionally  
2 provide an entrance well **312** at the entrance orifice **106B** for containing the sample fluid  
3 prior to entering the channel **104**. Although not shown, if the outer diameter of the capillary  
4 is less than that of the channel and the entrance orifice, the capillary may be inserted into and  
attached to the entrance orifice of the electrospray device.

5 Referring now to the schematic of FIG. 22, rather than a heterogeneous interface,  
6 a single integrated system **316** is provided wherein an upstream fluid delivery device **318**  
7 forms a homogeneous interface with the entrance orifice (not shown) of an electrospray  
8 device **100**. The system **316** allows for the fluid exiting the upstream fluid delivery device  
9 **318** to be delivered on-chip to the entrance orifice of the electrospray device **100** in order to  
generate an electrospray.

10 The single integrated system **316** provides the advantage of minimizing or  
11 eliminating extra fluid volume to reduce the risk of undesired fluid changes, such as by  
12 reactions and/or mixing. The single integrated system **316** also provides the advantage of  
13 eliminating the need for unreliable handling and attachment of components at the  
microscopic level and of minimizing or eliminating fluid leakage by containing the fluid  
within one integrated system.

14 The upstream fluid delivery device **318** may be a monolithic integrated circuit  
15 having an exit orifice through which a fluid sample can pass directly or indirectly to the  
16 entrance orifice of the electrospray device **100**. The upstream fluid delivery device **318** may  
17 be a silicon microchip-based liquid separation device capable of, for example, capillary  
18 electrophoresis, capillary electrochromatography, affinity chromatography, liquid  
19 chromatography (LC) or any other condensed-phase separation methods. Further, the  
20 upstream fluid delivery device **318** may be a silicon, glass, plastic and/or polymer based  
device such that the electrospray device **100** may be chip-to-chip or wafer-to-wafer bonded  
thereto by any suitable method. An example of a monolithic liquid chromatography device  
for utilization in, for example, the single integrated system **316**, is described below.

21 **Electrospray Device for Sample Transfer of Combinatorial Chemistry**  
22 **Libraries Synthesized in Microdevices**

23 The electrospray device may also serve to reproducibly distribute and deposit a  
24 sample from a mother plate to daughter plate(s) by nanoelectrospray deposition. Electrospray  
25 device(s) may be etched into a microdevice capable of synthesizing combinatorial chemical  
26 libraries. At the desired time, the nozzle may spray a desired amount of the sample from the  
mother plate to the daughter plate(s). Control of the nozzle dimensions, applied voltages,



1 and time of spraying may provide a precise and reproducible method of sample deposition  
2 from an array of nozzles, such as the generation of sample plates for molecular weight  
3 determinations by matrix-assisted laser desorption/ionization time-of-flight mass  
4 spectrometry (MALDI-TOFMS). The capability of transferring analytes from a mother plate  
5 to daughter plates may also be utilized to make other daughter plates for other types of  
assays, such as proteomic screening.

6 FIGS. 23A and 23B show; respectively, an exploded perspective view and a cross-  
7 sectional view along line 23B-23B, of a chip-based combinatorial chemistry system 320  
8 comprising a reaction well block or titer plate 322 and a receiving or daughter plate 324. The  
9 reaction well block 322 defines an array of reservoirs 326 for containing the reaction  
10 products from a combinatorially synthesized compound. The reaction well block 322 further  
11 defines channels 328, nozzles 330 and recessed portions 332 such that the fluid in each  
12 reservoir 326 may flow through a corresponding channel 328 and exit through a  
13 corresponding nozzle 330 in the form of an electrospray. The reaction well block 322 may  
14 define any number of reservoir(s) in any desirable configuration, each reservoir being of a  
suitable dimension and shape. The volume of a reservoir 326 may range from a few  
nanoliters up to several microliters and more preferably ranges between approximately 200  
nL to 1  $\mu$ L.

15 The reaction well block 322 may serve as a mother plate to interface to a microchip-  
16 based chemical synthesis apparatus such that the electrospray function of the reaction well  
17 block 322 may be utilized to reproducibly distribute discreet quantities of the product  
18 solutions to a receiving or daughter plate 324. The daughter plate 324 defines receiving  
19 wells 334 which correspond to each of the reservoirs 326. The distributed product solutions  
20 in the daughter plate 324 may then be utilized to screen the combinatorial chemical library  
against biological targets.

### 21 **Illustration of an Electrospray Device Generating an Electrospray Spray**

22 FIGS. 24A and 24B show color images of a real Taylor cone emanating from an  
23 integrated silicon chip-based nozzle. FIGS. 24C and 24D are perspective and side cross-  
24 sectional views, respectively, of the electrospray device and mass spectrometer system shown  
25 in FIGS. 24A and 24B. FIGS. 24A shows a chip-integrated electrospray device comprising  
26 a nozzle and a recessed portion or annulus, and a Taylor cone, liquid jet and plume of highly-  
charged electrosprayed droplets of methanol containing 10  $\mu$ g/mL polypropylene glycol 425  
(PPG425) containing 0.2% formic acid. FIG. 24B shows an ion-sampling orifice of a mass  
spectrometer in addition to the electrospray device.

1 The electrospray device 100 is interfaced upstream with a pipette 52'. As shown in  
2 the upper right corner of each of FIGS. 24A and 24B and in FIGS. 24C and 24D, the tip of  
3 the pipette 52' is press-sealed to the injection side of the electrospray device 100. The  
4 electrospray device 100 has a 10  $\mu\text{m}$  diameter entrance orifice on the injection side, a 30  $\mu\text{m}$   
5 inner diameter and a 60  $\mu\text{m}$  outer diameter nozzle, a 15  $\mu\text{m}$  nozzle wall thickness and a 150  
6  $\mu\text{m}$  nozzle depth. The recessed portion or the annulus extends 300 $\mu\text{m}$  from the outer  
7 diameter of the nozzle. The voltage applied to the fluid  $V_{\text{fluid}}$  introduced to the electrospray  
8 device and thus the nozzle voltage is 900 V. The voltage applied to the substrate  $V_{\text{substrate}}$  and  
9 thus the electrospray device is 0 V. The voltage applied to the mass spectrometer which also  
10 serves as an extracting electrode  $V_{\text{extract}}$  is approximately 40 V. The liquid sample was  
11 pumped using a syringe pump at a flow of 333 nL/min through the pipette tip pressed-sealed  
12 against the injection side of the electrospray device. The nozzle is approximately 5 mm from  
13 the ion-sampling orifice 62 of the mass spectrometer 60. The ion-sampling orifice 62 of the  
14 mass spectrometer 60 generally defines the acceptance region of the mass spectrometer 60.  
15 The mass spectrometer for acquiring the data was the LCT Time-Of-Flight mass  
16 spectrometer of Micromass, Inc.

17 FIG. 24E shows a mass spectrum of 1  $\mu\text{g/mL}$  PPG425 in 50% water, 50% methanol  
18 containing 0.1% formic acid, 0.1% acetonitrile and 2 mM ammonium acetate. The data were  
19 collected at a flow rate of 333 nL/min.

## 20 LIQUID CHROMATOGRAPHY DEVICE

21 In another aspect of the invention shown in the exploded perspective and cross-  
22 sectional views of FIGS. 25A and 25B, respectively, a silicon-based liquid chromatography  
23 device 400 generally comprises a silicon substrate or microchip 402 defining an introduction  
24 channel 404 through the substrate 402 extending between an entrance orifice 406 on a first  
25 surface 408 and a fluid reservoir 410, a separation channel 412 extending between the  
26 reservoir 410 and an exit orifice 414, a plurality of separation posts 416 along the separation  
channel 412, and a cover 420 to provide an enclosure surface adjacent the cover 420 for the  
reservoir 410 and the separation channel 412 adjacent the cover 420.

The plurality of separation posts 416 extends from a side wall of the separation  
channel 412 in a direction perpendicular to the fluid flow through the separation channel 412.  
Preferably, one of the ends of each separation post 416 does not extend beyond and is  
preferably coplanar or level with the second surface 417. The separation channel 412 is  
functionally similar to the liquid chromatography column in that component separation  
occurs in the separation channel 412 where the plurality of separation posts 416 perform the

liquid chromatography function. Component separation occurs through the interaction of the fluid flowing through the separation channel 412 wherein the columnar separation posts 416 provides the large surface area. The surfaces of the separation channel 412 and the separation posts 416 are preferably provided with an insulating layer to insulate the fluid in the separation channel 412 from the substrate 402. Specifically, the separation posts 416 are preferably oxidized silicon posts which may be chemically modified using known techniques in order to optimize the interaction of the components of the sample fluid with the stationary phase, the separation posts 416. In one embodiment, the separation channel 412 extends beyond the separation posts 416 to the edge of the substrate 402 and terminating as the exit orifice 414.

The introduction channel, **404**, the separation channel **412**, the reservoir **410** and the separation posts **416** may have any suitable cross-sectional shapes such as circular and/or rectangular. Preferably, the separation posts **416** have the same cross-sectional shapes and sizes but may nonetheless have different cross-sectional shapes and/or sizes.

The liquid chromatography device **400** further comprises a layer of silicon dioxide **422** over the surfaces of the substrate of the cover **420** and a layer of silicon dioxide **424** over the surfaces of the substrate **402**. The silicon dioxide layers **422**, **424** electrically isolate a fluid contained in the reservoir **410** and the separation channel **412** from the substrate **402** and the substrate of the cover **420**. The silicon dioxide layers **422**, **424** are also relatively inactive and thus less likely to interact with fluids in the reservoir **410** and the separation channel **412** than bare silicon.

Depending on the specific application, the substrate **402** may provide a surface on which one or more conductive electrodes in electrical contact with the fluid in the device **400** may be formed. For example, a reservoir electrode **426** and/or an exit electrode **428** may be provided on the second surface **417** of the substrate **402** such that a corresponding electrode would be in electrical contact with fluid in the reservoir **410** and near the exit orifice **414**, respectively. A filling electrode **430** may also be provided on the second surface **417** of the substrate **402** such that it would be in electrical contact with fluid in the unpopulated portion **432** of the separation channel **412** between the reservoir **410** and the first occurrence of separation posts **416**. The shape, size and location along the fluidic flow path of each electrode on the substrate **402** may be determined by design considerations such as the distance between adjacent electrodes. Further, any or all of the electrodes may be alternatively or additionally formed on the bonding surface **425** of the cover **420**. For example, the filling electrode **430** may be alternatively positioned such that it would be in

1 electrical contact with fluid in the separation channel **412** adjacent the reservoir **410**.  
2 Further, additional electrodes may be provided, for example, to create an arbitrary electrical  
3 potential distribution along the fluidic flow path.

4 Providing two or more of the reservoir, filling and exit electrodes along with  
5 electrical isolation of the fluid sample in the device **400** from the substrate **402** and the  
6 substrate of the cover **420** allows for the application and sustenance of different (or same)  
7 electric potentials at two or more different locations along the fluidic path. The difference  
8 in electric potentials at two or more different locations along the fluidic path causes fluidic  
9 motion to occur between the two or more locations. Thus, these electrodes may facilitate the  
10 filling of the reservoir **410** and/or the driving of the fluid through the separation channel **412**.

11 Further, through appropriate layout design and fabrication processes, the substrate  
12 **402** and/or the cover **420** may also provide additional functionalities such as pre-conditioning  
13 of the fluid prior to delivery into the reservoir **410**, and/or conveying, analyzing, and/or  
14 otherwise treating fluidic samples exiting from the separation channel **412**. The cover **420**  
15 may provide such additional functionality on either or both surfaces and/or the bulk of the  
16 cover **420**.

17 The cover **420** may comprise a substrate **418** comprising silicon or any other suitable  
18 material, such as glass, plastics and/or polymers. The specific material for the cover **420** may  
19 depend upon, for example, whether direct observation of a fluoresced fluid is desired such  
20 that glass may be more desirable and/or the consideration of the ease of fabrication of the  
21 cover **420** by utilizing similar processing techniques as for the substrate **402** such that silicon  
22 may be more desirable. The cover **420** may be bonded or otherwise affixed to form a  
23 hermetic seal between the substrate **402** and the cover **420** in order to ensure the appropriate  
24 level of fluid containment and isolation. For example, several methods of bonding silicon  
25 to silicon or glass to silicon are known in the art, including anodic bonding, sodium silicate  
26 bonding, eutectic bonding, and fusion bonding. The specific hermetic bonding method may  
depend on various factors such as the physical form of the surfaces of the substrate **402** and  
the cover **420** and/or the application and functionality of the integrated system and/or the  
liquid chromatography device **400**.

Dimensions of the liquid chromatography device **400** may be determined according  
to various factors such as the specific application, the layout design as well as the device with  
which it is to be interfaced or integrated. The surface dimensions, i.e. the dimensions in the  
X and Y directions, of the elements of the liquid chromatography device **400** may be  
determined by layout design and through the corresponding photomasks used in fabrication.

1 The depth or height, i.e. the dimension in the Z direction, of the elements of the liquid  
2 chromatography device **400** may be determined by the etch processes during fabrication, as  
3 described below. The depth or height of the elements is independent of the surface  
4 dimensions to a first-order approximation although the aspect ratio limitations of the  
5 reactive-ion etch places constraints on the etch depth, particularly with the small surface  
openings in the channel **412** between the separation posts **416**.

6 Further, the size, number, cross-sectional shape, spacing and placement of the  
7 separation posts **416** may also be determined by layout design to achieve the desired flow  
8 rate and to prevent low-resistance lines of sight within the separation channel **412** to ensure  
9 adequate fluid-surface interaction. Each separation post **416** may have the same or different  
10 characteristics such as size and/or cross-sectional shape. The cross-sectional shape of the  
11 posts may be chosen in layout design to optimize fluid/boundary layer interactions at the post  
12 surfaces. The separation posts **416** may be placed in any desired pattern in the separation  
13 channel **412**, such as periodic, semi-periodic, or random. Close spacing of the separation  
14 posts **416** may be desirable for maximization of the surface interactions with the fluid.  
15 Similarly, minimizing the cross-sectional area of the separation posts **416** may permit  
16 placement of greater number in the separation channel **412**. However, the reduction of the  
17 cross-sectional area of the separation posts **416** is limited by the resulting reduction in the  
18 mechanical stability necessary during processing.

19 Control of the size, number, cross-sectional shape, spacing and placement of the  
20 separation posts **416** provides advantages over traditional liquid chromatography as the  
21 traditional separation column packing materials have undesired dispersion in size distribution  
22 as well as random spacing variations.

23 In one currently preferred embodiment, the substrate **402** of the liquid  
24 chromatography device **400** is approximately 250-600  $\mu\text{m}$  in thickness, the separation  
25 channel **412** has a depth of approximately 10  $\mu\text{m}$ , the rectangular reservoir **410** is  
26 approximately 1000  $\mu\text{m}$  by 1000  $\mu\text{m}$  resulting in a volume of approximately 10 nL. The  
depth of the reservoir **410** and the separation channel **412** is limited by the height of the  
separation posts **416** which is in turn limited by the maximum etch aspect ratio. The nearest-  
neighbor spacing of the separation posts **416** is preferably less than approximately 5  $\mu\text{m}$ .  
The dimensions of the reservoir **410** determine the volume of the fluid sample which can be  
used for the liquid chromatography separation and, as is evident, through the independent  
control of surface dimensions and the depth, the reservoir **410** may be designed to have any  
desired volume. Preferably, the diameter of the entrance orifice **406** is 100  $\mu\text{m}$  or less such

1 that the fluid surface tension would be sufficient to maintain the fluid in the reservoir **410**  
2 to prevent leakage therefrom.

3 The silicon-based liquid chromatography device **400** reduces the size of a typical  
4 liquid chromatography device by nearly two orders of magnitude. The dimensional scaling  
5 may provide the advantage of significantly reducing the mass of the analyte and/or the  
6 volume of the fluid sample required for accurate analysis. Further, by reducing a  
macroscopic separation column and its packing materials to a monolithic device, the liquid  
chromatography device **400** can be a component of an on-chip integrated system.

7 Further, all features such as the reservoir, the separation channel and the separation  
8 posts are recessed from the substrate **402**. The portion of the substrate **402** exterior to the  
9 reservoir and the separation channel thus serves to physically protect the separation posts  
10 from casual abrasion and stress fracture in handling and subsequent bonding of the substrate  
11 **402** and the cover **420**. Because the posts are integral with the substrate, the posts are  
12 inherently stable and thus allow for the use of a pressurized system without the risk of  
damage to the stationary phase which may otherwise result with the use of conventional  
packing materials in conventional high-performance liquid chromatography systems.

13 An upstream fluid delivery system, such as a micropipette, piezoelectric pipette or  
14 small capillary, may be press-sealed onto the exterior surface of the liquid chromatography  
15 device **400** such that the pipette or capillary is concentric with the entrance orifice **406**.  
16 Optionally, the liquid chromatography device may provide a collar (not shown) to facilitate  
17 the mating and affixing of the fluid delivery device to the liquid chromatography device  
similar to the mating collar of the electrospray device as discussed with reference to FIG.  
21B.

18 To operate the liquid chromatography device **400**, the fluid reservoir **410** may first  
19 be filled with a sample fluid by injecting the fluid from a fluid delivery device through the  
20 introduction channel **404** via the entrance orifice **406**. Any suitable fluid delivery device  
21 such as a micropipette, a piezoelectric pipette or a small capillary may be utilized. The  
22 volume of the sample fluid injected into the liquid chromatography device **400** may be up  
23 to approximately the volume of the reservoir **410** plus a relatively small volume remaining  
in the introduction channel **404**.

24 The filling of the reservoir **410** may be facilitated by applying an appropriate  
25 potential voltage difference between the reservoir electrode **426** and the filling electrode **430**,  
26 such as approximately 1000 V/cm of introduction channel **404**. In particular, a volume of  
the fluid is first introduced into the reservoir **410** through the introduction channel **404** via

1 the entrance orifice 406 to coat or prime the surfaces of the reservoir 410 and the  
2 introduction channel 404 by capillary action to allow for electrical contact between the fluid  
3 and the reservoir and filling electrodes 426, 430. Where the filling electrode 430 is  
4 positioned in a portion of the separation channel 412 unpopulated by separation posts 416,  
5 the filling electrode 430 also facilitates the filling of the portion of the channel 412 between  
6 the reservoir 410 and the filling electrode 430.

6 After filling the reservoir 410 with an appropriate volume of the sample fluid, any  
7 suitable method may then be utilized to drive the fluid from the reservoir 410 into the  
8 separation channel 412. For example, the fluid may be driven from the filled reservoir 410  
9 through the separation channel 412 by applying hydrostatic pressure to the reservoir 410 via  
10 the entrance orifice 406.

9 Alternatively or additionally, the fluid may be driven through the separation channel  
10 412 by applying a suitable electrokinetic potential voltage difference between the reservoir  
11 electrode 426 and the exit electrode 428 to generate electrophoretic or electroosmotic fluidic  
12 motion. Preferably, the electric potential difference is approximately 1000 V/cm of  
13 separation channel length. Of course, any other suitable methods of inducing fluidic motion  
14 may be utilized. Pressure-driven and voltage-driven flow effect different separation  
15 efficiencies. Thus, depending upon the application, one or both may be utilized.

15 Fluid then exits from the separation channel 412 through the exit orifice 414 to, for  
16 example, a capillary 434, which has an off-chip interconnection with the exit orifice 414, as  
17 shown in FIG. 26. Alternatively, as shown in FIG. 27, the liquid chromatography device 400  
18 may perform separation on the fluid from reservoir 410 such that selected analytes from the  
19 separation performed by posts 416 passes through unpopulated channel 436 to another on-  
20 chip device 438, such as for analysis and/or mixing, while the remainder of the fluid is  
21 directed to the waste reservoir 439. The unpopulated channel 436 may be a mere  
22 continuation of the separation channel 412 of the liquid chromatography device 400 or a  
23 channel separate from the separation channel 412.

21 Two or more fluid samples may be driven through the liquid chromatography device  
22 400 by successively filling the reservoir and driving the fluid through the separation channel  
23 412. For example, in certain applications, it may be desirable or necessary to first coat the  
24 surfaces of the separation posts 416 with one or more reagents and then pass an analyte  
25 sample over the conditioned separation posts 416.

25 Various modifications may be made to the liquid chromatography device describe  
26 above. For example, as shown in FIG. 28, rather than defining the entrance orifice and the

1 introduction channel in the substrate, the liquid chromatography device **400'** may provide  
2 an introduction channel **404'** in the cover **420'** such that the entrance orifice **406'** is defined  
3 on an exterior surface of the cover **420'**. Further, the cover **420'** may define an exit channel  
4 **413** between an exit orifice **414'** defined on an exterior surface of the cover **420'** and a  
separation channel **412'** which terminates within the substrate **402'**.

5 In another variation, an additional introduction channel **440** and entrance orifice **442**  
6 may be defined in the substrate **402''**, as shown in FIG. 29, or in the cover (not shown). The  
7 additional introduction channel **440** introduces fluid to the separation channel **412''** such that  
8 the fluid from the additional introduction channel **440** intersects the path of fluid flow from  
9 the reservoir **410** through the unpopulated portion **432''** of the separation channel **412''**. The  
10 fluid reservoir **410** may be utilized as a buffer for an eluent and the additional introduction  
11 channel **440** may be utilized to introduce the fluid sample to the separation channel **412''**.  
12 Further, the additional entrance orifice **442** may be utilized to introduce several fluid samples  
13 in succession into the separation channel **412''**. For example, in certain applications, it may  
14 be necessary to first coat the surfaces of the separation posts **416** with one reagent and then  
15 pass an analyte over the conditioned surfaces of the separation posts **416**.

16 Referring now to FIGS. 30-35, although the liquid chromatography device has been  
17 described as comprising a single reservoir and a single separation channel, the monolithic  
18 liquid chromatography device may be easily adapted and modified to comprise multiples of  
19 the liquid chromatography device and/or multiple entrance orifices, exit orifices, reservoirs  
20 and/or separation channels. In each of the variations, any or all of the reservoir(s), separation  
21 channel(s), and separation posts may have different dimensions and/or shapes.

22 For example, multiple reservoir-separation channel combinations may be provided  
23 on a single chip. In particular, as shown in FIG. 30, a reservoir **410A** may feed into a  
24 separation channel **412A** having separation posts **416A** and another reservoir **410B** may feed  
25 into another separation channel **412B** having separation posts **416B**.

26 In another variation as shown in FIG. 31, a single reservoir **410C** may feed multiple  
separation channels **412C**, **412D**. Each of separation channels **412C**, **412D** may have therein  
separation posts **416C**, **416D**, respectively, which may have the same or different properties,  
such as number, size and shape. Another channel **412E** may be provided as a null channel  
completely unpopulated by separation posts. The output from the null channel **412E** may  
be utilized as a basis of comparison to the output from the separation channel(s) populated  
by separation posts. Alternatively, all of the channels **412C**, **412D**, **412E** may be separation  
channels having separation posts.



1 Referring now to FIG. 32, fluid from multiple reservoirs **410E** and **410F** may feed  
2 into a single separation channel **412F** via connecting channels **444E**, **444F**, respectively. The  
3 connecting channels **444E**, **444F** are preferably unpopulated by separation posts to facilitate  
4 the mixing of the fluid samples from the reservoirs **410E**, **410F** prior to passage through the  
5 separation channel **412F**. The mixing of samples may be utilized to condition the primary  
6 sample of interest prior to separation or to effect a reaction between the samples prior to  
7 passage through the populated portion of the separation channel **412F**. Alternatively, fluid  
8 such as a conditioning fluid from one reservoir **410E** may flow through the separation  
9 channel **412F** in order to condition the surfaces of the separation posts **416F** prior to the  
10 passage of the other sample such as an analyte sample from the other reservoir **410F**.  
11 Although the separation posts **416F** are shown as having different cross-sections, separation  
12 posts **416F** may have the same size and cross-sectional shape.

13 Alternatively, in addition to having fluid from multiple reservoirs feed into a single  
14 separation channel via connecting channels, fluid from another reservoir may be introduced  
15 to the fluid flow along the separation channel, before and/or after the fluid has passed  
16 through the populated portion of the separation channel. For example, FIG. 33 shows that  
17 the fluid from multiple reservoirs **410G**, **410H** may be fed into a single separation channel  
18 **412G** via connecting channels **444G**, **444H**, respectively, and fluid from another reservoir  
19 **410I** may be introduced to the fluid flow along the separation channel **412G** after the fluid  
20 has passed the separation posts **416G**. FIG. 34 shows that the fluid from multiple reservoirs  
21 **410J**, **410K** may be fed into a single separation channel **412J** via connecting channels **444J**,  
22 **444K**, respectively, and fluid from another reservoir **410L** may be introduced to the fluid  
23 flow along the separation channel **412J** prior to the fluid passing the separation posts **416J**.

24 For devices having multiple reservoirs and/or multiple channels, separate electrodes  
25 may be provided for each reservoir and/or for each channel, for example, in the unpopulated  
26 portion of the channel upstream from the separation posts and/or near the exit of the channel.  
Such provision of separate electrodes allow for the separate and independent control of the  
fluidic flow for filling each reservoir and/or for driving the fluid through the separation  
channel.

The electric control may be simplified by having one common reservoir electrode,  
one common filling electrode, and/or one exit electrode among the multiple reservoirs and/or  
multiple channels. For example, each of the multiple reservoirs may be separately filled by  
applying a first voltage to the common reservoir electrode and a second voltage, different  
from the first voltage, to the filling electrode corresponding to the reservoir to be filled while

1 applying the first voltage to each of the other filling electrodes. As is evident, the multiple  
2 reservoirs may be simultaneously filled by applying a first voltage to the common reservoir  
3 electrode and a second, different voltage to each of the filling electrodes. Similarly, fluid  
4 may be separately driven through each of the multiple channels by applying a third voltage  
5 to the common reservoir electrode while applying a fourth voltage, different from the third  
6 voltage, to the exit electrode corresponding to the channel through which fluid is to be driven  
7 and the third voltage to each of the other exit electrodes.

8 In yet another variation shown in FIG. 35, in addition to a sample reservoir **410M**  
9 and separation posts **416M**, a plurality of posts **416L** may be provided in a channel **412M**  
10 upstream from the separation posts **416M** for providing additional functionality such as  
11 solid-phase extraction (SPE) for sample pretreatment. The SPE posts **416L** may be the same,  
12 similar to or different from the separation posts **416M** simply by varying the layout design.  
13 The SPE posts **416L** may provide surface functionality different from that of the separation  
14 posts **416M**. Alternatively, rather than providing a sample reservoir, an introduction channel  
15 (not shown) may be utilized to introduce a fluidic sample directly in the channel **412M** by  
16 allowing direct injection of the sample therein. Further, reservoirs **410N**, **410P** may be  
17 provided to contain fluidic buffers necessary for sample pretreatment upstream of the posts  
18 **416L**. For example, an eluent reservoir may be provided for eluting analytes and a wash  
19 reservoir may be provided for sample cleanup.

20 After the fluid samples pass the SPE posts **416L**, waste products from, for example,  
21 the solid-phase extraction process may be directed into a waste reservoir **410Q**. In particular,  
22 during the SPE process, voltage differences may be applied between or amongst reservoirs  
23 **410M**, **410N**, **410P**, and **410Q** such that a portion of the fluid from reservoirs **410M**, **410N**  
24 is directed to waste reservoir **410Q** while the remaining portion of the fluid from reservoir  
25 **410M** remain on the SPE posts **416L**. Material may then be washed off of the SPE posts  
26 **416L** by directing fluid from, for example, reservoir **410P** through channel **412M** for  
separation of the extracted material by separation posts **416M**. Additional reservoirs **410R**,  
**410S** downstream of the waste reservoir **410Q** and upstream of the separation posts **416M**  
may be provided to contain gradient elution of analytes in one reservoir and a diluent in the  
other reservoir. Gradient elution facilitates chromatography by changing the mobile phase  
composition, i.e. the polarity to facilitate analyte interactions with the stationary phase, and  
thus facilitate separation of the analytes. In addition, the diluent provides the correct polarity  
of the solution for the next separation.

# **LIQUID CHROMATOGRAPHY DEVICE FABRICATION PROCEDURE**

The fabrication of the liquid chromatography device of the present invention will now be explained with reference to FIGS. 36A-46B. The liquid chromatography device is preferably fabricated as a monolithic silicon micro device utilizing established, well-controlled thin-film silicon processing techniques such as thermal oxidation, photolithography, reactive-ion etching (RIE), ion implantation, and metal deposition. Fabrication using such silicon processing techniques facilitates massively parallel processing of similar devices, is time- and cost-efficient, allows for tighter control of critical dimensions, is easily reproducible, and results in a wholly integral device, thereby eliminating any assembly requirements. Manipulation of separate components and/or sub-assemblies to build an liquid chromatography device with high reliability and yield is not desirable and may not be possible at the micrometer dimensions required for efficient separation.

Further, the fabrication sequence may be easily extended to create physical aspects or features to facilitate interfacing, integration and/or connection with devices having other functionalities or to facilitate integration with a fluid delivery subsystem to create a single integrated system. Consequently, the liquid chromatography device may be fabricated and utilized as a disposable device, thereby eliminating the need for column regeneration and eliminating the risks of sample cross-contamination.

Referring to the plan and cross-sectional views, respectively, of FIGS. 36A and 36B, a silicon wafer separation substrate **500**, double-side polished and approximately 250-600  $\mu\text{m}$  in thickness, is subjected to an elevated temperature in an oxidizing ambient to grow a layer or film of silicon dioxide **502** on the reservoir side **503** and a layer or film of silicon dioxide **504** on the back side **505** of the separation substrate **500**. Each of the resulting silicon dioxide layers **502**, **504** has a thickness of approximately 1-2  $\mu\text{m}$ . The silicon dioxide layers **502**, **504** provide electrical isolation and also serve as masks for subsequent selective etching of certain areas of the separation substrate **500**.

A film of positive-working photoresist **506** is deposited on the silicon dioxide layer **502** on the reservoir side **503** of the separation substrate **500**. Certain areas of the photoresist **506** corresponding to the reservoir, separation channel and separation posts which will be subsequently etched are selectively exposed through a mask by an optical lithographic exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths of 365, 405, or 436 nanometers.

1 Referring to the plan and cross-sectional views, respectively, of FIGS. 37A and 37B,  
2 after development of the photoresist **506**, the exposed areas **508**, **509**, **510** of the photoresist  
3 corresponding to the reservoir, separation posts and channel, respectively, are removed and  
4 open to the underlying silicon dioxide layer **502** while the unexposed areas remain protected  
5 by photoresist **506'**. The exposed areas **508**, **509**, **510** of the silicon dioxide layer **502** are  
6 then etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to  
7 the protective photoresist **506'** until the silicon separation substrate **500** is reached. The  
8 remaining photoresist is removed in an oxygen plasma or in an actively oxidizing chemical  
9 bath like sulfuric acid ( $H_2SO_4$ ) activated with hydrogen peroxide ( $H_2O_2$ ).

10 As shown in the cross-sectional view of FIG. 38, the reservoir **410**, the separation  
11 channel **412**, and the separation posts **416** in the separation channel **412** are vertically formed  
12 in the silicon separation substrate **500** by another fluorine-based etch. Preferably, the  
13 reservoir **410** and the separation channel **412** have the same depth controlled by the etch time  
14 at a known etch rate. The simultaneous formation of the reservoir **410** and the channel **412**  
15 ensures uniform depth such that there are no discontinuities in the fluid-constraining surfaces  
16 to impede the fluid flow. The depth of the reservoir **410** and the channel **412** is preferably  
17 between approximately 5-20  $\mu m$  and more preferably approximately 10  $\mu m$ . The etch can  
18 reliably and reproducibly be executed to produce an aspect ratio (etch depth to width) of up  
19 to 30:1. Although not shown, any other reservoirs and/or channels, populated or  
20 unpopulated, may also be formed by this etch sequence.

21 A film of positive-working photoresist is then deposited over the silicon dioxide  
22 layer **502** and the exposed separation substrate **500** on the reservoir side **503** of the separation  
23 substrate **500**. An area of the photoresist corresponding to the introduction channel which  
24 will be subsequently etched is selectively exposed through a mask by an optical lithographic  
25 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths  
26 of 365, 405, or 436 nanometers. After development of the photoresist, the exposed area of  
the photoresist corresponding to the introduction channel is removed and open to the  
underlying separation substrate **500** while the unexposed areas remain protected by the  
photoresist.

As shown in the plan and cross-sectional views of FIGS. 39A and 39B, respectively,  
the exposed area of the separation substrate **500** is then vertically etched by a fluorine-based  
plasma with a high degree of anisotropy and selectivity to the protective photoresist until the  
silicon dioxide layer **504** on back side **505** is reached. Thus, a portion of the introduction  
channel **404** is formed through the separation substrate **500**. The remaining photoresist is

1 removed in an oxygen plasma or in an actively oxidizing chemical bath like sulfuric acid  
2 ( $\text{H}_2\text{SO}_4$ ) activated with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The silicon dioxide layer **504** on the back  
3 side **505** may then be removed by, for example, an unpatterned etch in a fluorine-based  
4 plasma.

5 Alternatively, as shown in FIGS. 40A and 40B, the introduction channel **404** may  
6 be formed by etching from both the reservoir side **503** and the back side **505** of the substrate  
7 **500**. After performing a vertical etch through a portion of the substrate **500** to form a portion  
8 of the introduction channel **404** in a manner similar to that described above, a film of  
9 positive-working photoresist **512** is deposited on the silicon dioxide layer **504** on the back  
10 side **505** of the separation substrate **500**. Patterns on the back side **505** may be aligned to  
11 those previously formed on the reservoir side **503** of the separation substrate **500**. Because  
12 silicon and its oxide are inherently relatively transparent to light in the infrared wavelength  
13 range of the spectrum, i.e. approximately 700-1000 nanometers, the extant pattern on the  
14 reservoir side **503** can be distinguished with sufficient clarity by illuminating the separation  
15 substrate **500** from the patterned reservoir side **503** with infrared light. Thus, the mask for  
16 the back side **505** can be aligned within required tolerances. Upon alignment, an area of the  
17 photoresist **512** corresponding to the entrance orifice and the introduction channel which will  
18 be subsequently etched is selectively exposed through a mask by an optical lithographic  
19 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths  
20 of 365, 405, or 436 nanometers.

21 After development of the photoresist **512**, the exposed area **514** of the photoresist  
22 corresponding to the entrance orifice is removed to expose the underlying silicon dioxide  
23 layer **504** on the back side **505** of the separation substrate **500** while the unexposed areas  
24 remain protected by the photoresist **512**. The exposed area **514** of the silicon dioxide layer  
25 **504** is then etched by a fluorine-based plasma with a high degree of anisotropy and  
26 selectivity to the protective photoresist **512** until the substrate **500** is reached. The remaining  
photoresist provides additional masking during a subsequent fluorine-based silicon etch to  
vertically etch the backside portion of the introduction channel. Thus, a through-substrate  
introduction channel **404** is complete. The remaining photoresist is removed in an oxygen  
plasma or in an actively oxidizing chemical bath like sulfuric acid ( $\text{H}_2\text{SO}_4$ ) activated with  
hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).

Preferably, the introduction channel **404** has the same diameter as the entrance  
orifice. A practical limit on etch aspect ratio of 30:1 constrains the diameter of the entrance  
orifice being etched to be approximately 10  $\mu\text{m}$  or greater for substrates of approximately

1 300  $\mu\text{m}$  thickness. Preferably, the entrance orifice **406** and the introduction channel **404**  
2 are approximately 100  $\mu\text{m}$  in diameter due to practical considerations. For example, the  
3 etch aspect ratio imposes a minimum diameter, and the diameter is preferably sufficiently  
4 large to enable ease of filling the reservoir **410** yet sufficiently small to ensure a fluid surface  
tension to prevent the fluid from leaking out of the reservoir **410**.

5 Alternatively, both the introduction channel and the entrance orifice may be formed  
6 by etching from the back side **505** of the separation substrate **500**. This may be preferable  
7 as it may be difficult to satisfactorily coat the separation posts **416** with photoresist. Further,  
8 this may be desirable depending on the application of the device, e.g. the external sample  
9 delivery system, the desired chip handling devices, the interfacing with other devices, chip-  
10 based or non-chip based, and/or the packaging considerations of the chip. Referring to the  
11 cross-sectional view of FIG. 41, after the reservoir, separation channel and the separation  
12 posts are etched in the separation substrate **500** (shown in FIG. 38), a film of positive-  
13 working photoresist **516** is deposited on the silicon dioxide layer **504** on the back side **505**  
14 of the separation substrate **500**. Patterns on the back side **505** may be aligned to those  
15 previously formed on the reservoir side **503** of the separation substrate **500** by illuminating  
16 the separation substrate **500** from the patterned reservoir side **503** with infrared light, as  
described above. Upon alignment, an area of the photoresist **516** corresponding to the  
entrance orifice which will be subsequently etched is selectively exposed through a mask by  
an optical lithographic exposure tool passing short-wavelength light, such as blue or near-  
ultraviolet at wavelengths of 365, 405, or 436 nanometers.

17 After development of the photoresist **516**, the exposed area **518** of the photoresist  
18 **516** corresponding to the entrance orifice is removed to expose the underlying silicon dioxide  
19 layer **504** on the back side **505** of the separation substrate **500**. The exposed area **518** of the  
20 silicon dioxide layer **504** is then etched by a fluorine-based plasma with a high degree of  
21 anisotropy and selectivity to the protective photoresist **512** until the silicon separation  
22 substrate **500** is reached. The remaining photoresist is left in place to provide additional  
23 masking during the subsequent etch through the silicon separation substrate **500**.

24 Referring now to the cross-sectional view of FIG. 42, the introduction channel **404**  
25 is vertically formed through the silicon separation substrate **500** by another fluorine-based  
26 etch. The introduction channel **404** is completed by etching through the separation substrate  
**500** until the reservoir **410** is reached. Thus, the introduction channel **404** extends through  
the separation substrate **500** between the entrance orifice **406** on the back side **505** of the  
separation substrate **500** and the reservoir **410**. The remaining photoresist is removed in an

1 oxygen plasma or in an actively oxidizing chemical bath like sulfuric acid ( $H_2SO_4$ ) activated  
2 with hydrogen peroxide ( $H_2O_2$ ).

### **Oxidation for surface passivation and fluid isolation**

3 As shown in the cross-sectional view of FIG. 43, a layer of silicon dioxide **522** is  
4 grown on all silicon surfaces of the substrate **500** by subjecting the silicon substrate **500** to  
5 elevated temperature in an oxidizing ambient. For example, the oxidizing ambient may be  
6 an ultra-pure steam produced by oxidation of hydrogen for a silicon dioxide thickness greater  
7 than approximately several hundred nanometers or pure oxygen for a silicon dioxide  
8 thickness of approximately several hundred nanometers or less. The layer of silicon dioxide  
9 **522** over all silicon surfaces of the separation substrate **500** electrically isolates a fluid in the  
10 channel from the silicon substrate **500** and permits the application and sustenance of an  
11 electric potential difference between the reservoir and the exit of the separation channel,  
12 between the reservoir and an unpopulated portion of the separation channel near the reservoir  
13 to facilitate in filling the reservoir and/or between other points along the fluid flow path.  
14 Thus, the application and sustenance of a significant voltage across the fluid sample may be  
15 achieved. Further, oxidation renders a surface inactive relative to a bare silicon surface,  
16 resulting in surface passivation.

17 All silicon surfaces are oxidized to form silicon dioxide with a thickness that is  
18 controllable through choice of temperature and time of oxidation. The final thickness of the  
19 silicon dioxide can be selected to provide the desired degree of electrical isolation in the  
20 device, where a thicker layer of silicon dioxide provides a greater resistance to electrical  
21 breakdown.

22 Photolithography and reactive-ion etching limit the layout design of separation post  
23 diameters and inter-post spacing to greater than approximately  $1\text{ }\mu\text{m}$ . However, because the  
24 thermal oxidation process consumes approximately  $0.44\text{ }\mu\text{m}$  of silicon to form each  
25 micrometer of silicon dioxide, the thermal oxidation process results in a volumetric  
26 expansion. This volumetric expansion may be utilized to reduce the spacing between the  
separation posts **416** to sub-micrometer dimensions. For example, with a layout inter-post  
spacing of approximately  $1.5\text{ }\mu\text{m}$ , oxidation producing a  $1\text{ }\mu\text{m}$  silicon dioxide film or layer  
would result in a nearest-neighbor spacing of approximately  $0.5\text{ }\mu\text{m}$ . Further, because the  
oxidation process is well-controlled, separation post dimensions, including the inter-post  
spacing, in the sub-micrometer regime can be formed reproducibly and in a high yielding  
manner.

1        FIGS. 44A, 44B and 44C show scanning electron microscope photographs and  
2        design layout of portions of fabricated liquid chromatography devices. FIG. 44A shows a  
3        design layout of a portion of a reservoir and separation posts in a portion of a separation  
4        channel where the separation posts have rectangular cross-sectional shape. FIG. 44B shows  
5        separation posts in a portion of a separation channel, the separation posts having a circular  
6        cross-sectional shape and a diameter and inter-post spacing of approximately 1  $\mu\text{m}$ . FIG. 44C  
7        shows separation posts in a portion of a separation channel, the separation posts having a  
8        rectangular or square cross-sectional shape with a dimension of 2  $\mu\text{m}$  and inter-post spacing  
9        of approximately 1  $\mu\text{m}$ .

10       In a variation, the entrance orifice and the introduction channel for filling the fluid  
11       reservoir may be formed in the cover substrate 524 after a layer of silicon dioxide 525 is  
12       grown on all surfaces of the cover substrate 524, rather than in the substrate 500. As shown  
13       in FIG. 45, the cover substrate 524 may be bonded to the reservoir side 503 of the separation  
14       substrate 500. The entrance orifice 406' and the introduction channel 404' may be formed  
15       in the cover substrate 524 after alignment with respect to the reservoir 410. The entrance  
16       orifice 406' and the introduction channel 404' may be formed in the same or similar manner  
17       as described above by utilizing lithography to define the entrance orifice pattern and reactive-  
18       ion etching to create the entrance orifice and the through-cover introduction channel. The  
19       cover substrate 524 is again subjected to elevated temperature in an oxidizing ambient to  
20       grow a layer of oxide on the surface of the introduction channel 404'. Further, the  
21       introduction channel 404' may be formed from one or two sides of the cover substrate 524.  
22       If channel 404' is formed from two sides of the cover substrate, the cover substrate 524 may  
23       be bonded to substrate 500 after forming the channel 404' and after oxidation of the channel  
24       surface. One advantage of defining the entrance orifice on the same side of the completed  
25       liquid chromatography device as the reservoir and separation channel is that the back side  
26       of the substrate 500 is then free from any features and may then be bonded to a protective  
27       package without special provision for filling the reservoir through an entrance orifice defined  
28       on the back-side of the substrate.

#### 29       **Metallization for fluid flow control**

30       FIGS. 46A and 46B illustrate the formation of a reservoir, a filling, and an exit  
31       electrode as well as conductive lines or wires connecting the electrodes to bond pads in the  
32       cover substrate 526, preferably comprising glass and/or silicon. The cover substrate 526  
33       shown in FIGS. 46A and 46B does not provide an entrance orifice or an introduction channel



1 although the metallization process described herein may be easily adapted for a cover  
2 substrate providing an entrance orifice and an introduction channel.

3 As shown in the plan and cross-sectional view of FIGS. 46A and 46B, respectively,  
4 prior to the depositing of conductive material on the cover substrate **526**, all surfaces of the  
5 cover substrate **526** are subjected to thermal oxidization in a manner that is the same as or  
6 similar to the process described above to create a film or layer of silicon dioxide **528**. Such  
7 oxidization is not performed where the cover substrate **526** comprises glass.

8 The silicon dioxide layer **528** provides a surface on which conductive electrodes may  
9 be formed. The thickness of the silicon dioxide layer **528** is controllable through the  
10 oxidation temperature and time and the final thickness can be selected to provide the desired  
11 degree of electrical isolation, where a thicker layer of silicon dioxide provides a greater  
12 resistance to electrical breakdown. The silicon dioxide layer **528** electrically isolates all  
13 electrodes from the cover substrate **526** and isolates the fluid in the reservoir and the channel  
14 of the liquid chromatography device from the cover substrate **526**. The ability to isolate the  
15 fluid from the cover substrate **526** complements the electrical isolation provided in the  
16 separation substrate through oxidation and ensures the complete electrical isolation of the  
17 fluid from both the separation substrate and the cover substrate **526**. The complete electrical  
18 isolation of the sample fluid from both substrates allows for the application of electric  
19 potential differences between spatially separated locations in the fluidic flow path resulting  
20 in control of the fluid flow through the path.

21 The cover substrate **528** may be cleaned after oxidation utilizing an oxidizing  
22 solution such as an actively oxidizing chemical bath, for example, sulfuric acid ( $H_2SO_4$ )  
23 activated with hydrogen peroxide ( $H_2O_2$ ). The cover substrate **528** is then thoroughly rinsed  
24 to eliminate organic contaminants and particulates. A layer of conductive material **530** such  
25 as aluminum is then deposited by any suitable method such as by DC magnetron sputtering  
26 in an argon ambient. The thickness of the aluminum is preferably approximately 3000 Å,  
although shown having a larger thickness for clarity. Although aluminum is utilized in the  
fabrication sequence described herein, any type of highly conductive material such as other  
metals, metallic multi-layers, silicides, conductive polymers, and conductive ceramics like  
indium tin oxide (ITO) may be utilized for the electrodes. The surface preparation for  
satisfactory adhesion may vary depending on the specific electrode material used. For  
example, the silicon dioxide layer **528** provides a surface to which aluminum electrodes may  
adhere as aluminum does not generally adhere well to native silicon.

Alternatively, a metal lead may be formed from each electrode to an otherwise unpatterned area of the separation substrate such that a through-substrate access channel formed in the cover substrate and filled with a conductive material by chemical vapor deposition (CVD) allows access to the electrode(s). As an alternative to chemical vapor deposition, the sidewalls of the through-substrate access channel may be sloped, for example by KOH etch, to facilitate continuous deposition of a conductive material thereon, thereby providing an electrically continuous path from the separation substrate to the top of the cover substrate where potential voltages can be applied. In these variations, the separation and the cover substrates may be of the same size.

1 Although the electrodes are preferably provided on a surface of the cover substrate,  
2 the electrodes may be alternatively and/or additionally provided on the separation substrate  
3 by appropriate modifications to the above-described fabrication process. For example, in  
4 such a variation, the side walls of the reservoir are preferably not at a 90° angle relative to  
5 the bottom wall and can be formed at least in part by, for example, a wet chemical potassium  
6 hydroxide (KOH) etch. The sloped reservoir side walls allow for the deposition of a  
7 conductive material thereon. In another variation, the electrodes may also be formed by a  
8 damascene process, known in the art of semiconductor fabrication. The damascene process  
9 provides the advantage of a planar surface without the step up and step down surface  
10 topography presented by a bond line or pad and thus facilitates the bonding of the separation  
11 and cover substrate, as described below.

12 The above described fabrication sequence for the liquid chromatography device may  
13 be easily adapted to and is applicable for the simultaneous fabrication of a monolithic system  
14 comprising multiple liquid chromatography devices including multiple reservoirs and/or  
15 multiple separation channels as described above embodied in a single monolithic substrate.

16 Further, although the fabrication sequence is described in terms of fabricating a  
17 single liquid chromatography device, the fabrication sequence facilitates and allows for  
18 massively parallel processing of similar devices. The multiple liquid chromatography  
19 devices or systems fabricated by massively parallel processing on a single wafer may then  
20 be cut or otherwise separated into multiple devices or systems.

21 Although control of the liquid chromatography device has been described above as  
22 comprising reservoir, filling and exit electrodes, any suitable combination of such and/or  
23 other electrodes in electrical contact with the fluid in the fluid path may be provided and  
24 easily fabricated by modifying the layout design. Further, any or all of the electrodes may  
25 be additionally or alternatively provided in the separation substrate. Electrodes may be  
26 formed in the separation substrate by modifying the fabrication sequence to include  
additional steps similar to or the same as the steps as described above with respect to the  
formation of the electrodes in the cover substrate.

#### **Bonding cover substrate to separation substrate**

As described above, the cover substrate is preferably hermetically bonded by any  
suitable method to the separation substrate for containment and isolation of the fluid in the  
liquid chromatography device. Examples of bonding silicon to silicon or glass to silicon  
include anodic bonding, sodium silicate bonding, eutectic bonding, and fusion bonding.

1 For example, to bond the separation substrate to a glass cover substrate by anodic  
2 bonding, the separation substrate and cover substrate are heated to approximately 400°C and  
3 a voltage of 400-1200 Volts is applied, with the separation substrate chosen as the anode (the  
4 higher potential). Further, as the required bonding voltage depends on the surface oxide  
5 thickness, it may be desirable to remove the oxide film or layer from the back side 505 of the  
6 separation substrate prior to the bonding process in order to reduce the required bonding  
7 voltage. The oxide film or layer may be removed by, for example, an unpatterned etch in a  
8 fluorine-based plasma. The etch is continued until the entire oxide layer has been removed,  
9 and the degree of over-etch is unimportant. Thus, the etch is easily controlled and high-  
10 yielding.

11 Critical considerations in any of the bonding methods include the alignment of  
12 features in the separation and the cover substrates to ensure proper functioning of the liquid  
13 chromatography device after bonding and the provision in layout design for conductive lead-  
14 throughs such as the bond pads and/or metal lines so that the electrodes (if any) are  
15 accessible from outside the liquid chromatography device. Another critical consideration is  
16 the topography created through the fabrication sequence which may compromise the ability  
17 of the bonding method to hermetically seal the separation and cover substrates. For example,  
18 the step up and step down in the surface topography presented by a metal line or pad may be  
19 particularly difficult to form a seal therearound as the silicon or glass does not readily deform  
20 to conform to the shape of the metal line or pad, leaving a void near the interface between  
21 the metal and the oxide.

## 22 **INTEGRATION OF LIQUID CHROMATOGRAPHY AND ELECTROSPRAY** 23 **DEVICES ON A CHIP**

24 The cross-sectional schematic view of FIG. 47 shows a liquid chromatography-  
25 electrospray system 600 comprising a liquid chromatography device 602 of the present  
26 invention integrated with an electrospray device 620 of the present invention such that a  
homogeneous interface is formed between the exit orifice 614 of the liquid chromatography  
device 602 and the entrance orifice 622 of the electrospray device 620. The single integrated  
system 600 allows for the fluid exiting the exit orifice 614 of the liquid chromatography  
device 602 to be delivered on-chip to the entrance orifice 622 of the electrospray device 620  
in order to generate an electrospray.

As shown in FIG. 47, the entrance orifice 606 and the introduction channel 604

1 of the liquid chromatography device 602 are formed in the cover substrate 608 along with  
2 the electrospray device 620. Alternatively, the liquid chromatography entrance orifice and  
the introduction channel may be formed in the separation substrate.

3 Fluid at the electrospray nozzle entrance 622 is at the exit voltage applied to the exit  
4 electrode 610 in the separation channel 612 near the liquid chromatography exit orifice 614.  
5 Thus, an electrospray entrance electrode is not necessary.

6 The single integrated system 600 provides the advantage of minimizing or  
eliminating extra fluid volume to reduce the risk of undesired fluid changes, such as by  
7 reactions and/or mixing. The single integrated system 600 also provides the advantage of  
8 eliminating the need for unreliable handling and attachment of components at the  
microscopic level and of minimizing or eliminating fluid leakage by containing the fluid  
9 within one integrated system.

10 The integrated liquid chromatography-electrospray system 600 may be utilized to  
11 deliver liquid samples to the sampling orifice of a mass spectrometer. The sampling orifice  
12 of the mass spectrometer may serve as an extraction electrode in the electrospray process  
when held at an appropriate voltage relative to the voltage of the electrospray nozzle 624.  
13 The liquid chromatography-electrospray system 600 may be positioned within 10 mm of the  
14 sampling orifice of the mass spectrometer for efficient extraction of the fluid from the  
electrospray nozzle 624.

#### 15 **Multiple liquid chromatography-electrospray systems on a single chip**

16 Multiples of the liquid chromatography-electrospray system 600 may be formed on  
17 a single chip to deliver a multiplicity of samples to a common point for subsequent  
sequential analysis. For example, FIG. 48 shows a plan view of multiple liquid  
18 chromatography-electrospray systems 600 on a single chip 650 and FIG. 49 shows a detailed  
19 view of area A of systems 600 with the separation channels shown in phantom and without  
the recessed portions for purposes of clarity. As shown, the multiple nozzles 624 of the  
20 electrospray devices 620 may be radially positioned about a circle having a relatively small  
21 diameter near the center of the single chip 650. The dimensions of the electrospray nozzles  
and the liquid chromatography channels limit the radius at which multiple nozzles are  
22 positioned on the multi-system chip 650. For example, the multi-system chip may provide  
23 96 nozzles with widths of up to 50  $\mu\text{m}$  positioned around a circle 2 mm in diameter such that  
24 the spacing between each pair of nozzles is approximately 65  $\mu\text{m}$ .

25 Alternatively, an array of multiple electrospray devices without liquid  
26 chromatography devices may be formed on a single chip to deliver a multiplicity of

1 samples to a common point for subsequent sequential analysis. The nozzles may be similarly  
2 radially positioned about a circle having a relatively small diameter near the center of the  
3 chip. The array of electrospray devices on a single microchip may be integrated upstream  
4 with multiple fluid delivery devices such as separation devices fabricated on a single  
5 microchip. For example, an array of radially distributed exit orifices of a radially distributed  
6 array of micro liquid chromatography columns may be integrated with radially distributed  
7 entrance orifices of electrospray devices such that the nozzles are arranged at a small radius  
8 near the orifice of a mass spectrometer. Thus, the electrospray devices may be utilized for  
9 rapid sequential analysis of multiple sample fluids. However, depending upon the specific  
10 application and/or the capabilities of the downstream mass spectrometer (or other  
11 downstream device), the multiples of the electrospray devices may be utilized one at a time  
or simultaneously, either all or a portion of the electrospray devices, to generate one or more  
electrosprays. In other words, the multiples of the electrospray devices may be operated in  
parallel, staggered or individually.

12 The single multi-system chip 650 may be fabricated entirely in silicon substrates,  
13 thereby taking advantage of well-developed silicon processing techniques described above.  
14 Such processing techniques allow the single multi-system chip 650 to be fabricated in a cost-  
15 effective manner, resulting in a cost performance that is consistent with use as a disposable  
16 device to eliminate cross-sample contamination. Furthermore, because the dimensions and  
17 positions of the liquid chromatography-electrospray systems are determined through layout  
design rather than through processing, the layout design may be easily adapted to fabricate  
multiple liquid chromatography-electrospray systems on a single chip.

#### 18 **Interface of a multi-system chip to mass spectrometer**

19 The radially distributed array of electrospray nozzles 624 on a multi-system chip  
20 may be interfaced with a sampling orifice of a mass spectrometer by positioning the nozzles  
21 near the sampling orifice. The tight radial configuration of the electrospray nozzles 624  
allows the positioning thereof in close proximity to the sampling orifice of a mass  
spectrometer.

22 The multi-system chip 650 may be rotated relative to the sampling orifice to position  
23 one or more of the nozzles for electrospray near the sampling orifice. Appropriate voltage(s)  
24 may then be applied to the one or more of the nozzles for electrospray. Alternatively, the  
multi-system chip 650 may be fixed relative to the sampling orifice of a mass spectrometer  
25 such that all nozzles, which converge in a relatively tight radius, are appropriately  
26 positioned for the electrospray process. As is evident, eliminating the need for nozzle

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What is claimed and desired to be secured by United States Letters Patent is:

- 1           1.     An integrated miniaturized system for chemical analysis of fluids, comprising:  
2                 an electrospray substrate having an injection side and an ejection surface, the  
3                 substrate defining an entrance orifice on the injection side, a nozzle on the ejection  
4                 surface, a channel extending between the entrance orifice and the nozzle, and a  
5                 region surrounding the nozzle recessed from the ejection surface; and  
6                 electrode means for providing electrical contact to the fluids.
- 7           2.     The system of claim 1, wherein said electrode means comprises an external  
8                 conductor in contact with the fluid prior to said injection side.
- 9           3.     The system of claim 1, wherein the channel has a cross-sectional area less than  
10                 approximately 50,000  $\mu\text{m}^2$ .
- 11           4.     The system of claim 1, wherein the substrate defines a plurality of entrance  
12                 orifices on the injection side, a plurality of nozzles on the ejection surface each  
13                 corresponding to one of the plurality of entrance orifices, a plurality of channels each  
14                 extending between one of the plurality of nozzles and the corresponding one of the plurality  
15                 of entrance orifices.
- 16           5.     The system of claim 4, wherein an array of said plurality of nozzles are radially  
17                 positioned on the ejection surface of the electrospray substrate.
- 18           6.     The system of claim 1, further comprising a device in fluid communication with  
19                 the entrance orifice.
- 20           7.     The system of claim 1, wherein an array of nozzles are defined on the ejection  
21                 surface of the electrospray substrate, and further comprising a daughter plate defining a  
22                 plurality of receiving wells positioned to receive a fluid ejected through the nozzles of the  
23                 electrospray substrate.
- 24           8.     The system of claim 1, further comprising a second substrate defining an  
25                 entrance opening on a first surface and an exit on a second surface, the second substrate  
26                 being bonded to the electrospray substrate such that the second substrate exit is in fluid  
                communication with the electrospray substrate entrance orifice.
9.     The system of claim 1, further comprising a second substrate defining an  
                entrance opening on a first surface, an exit on a second surface, a fluid reservoir recessed  
                from the second surface, a separation channel recessed from the second surface, the  
                separation channel including the exit and extending between the reservoir and the exit, an  
                introduction channel extending between the entrance opening and the reservoir, and a  
                plurality of posts extending from the separation channel, wherein the second substrate is  
                bonded to the electrospray substrate to enclose the reservoir and the separation channel



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adjacent the electrospray substrate and such that the second substrate exit is in fluid communication with the electrospray substrate entrance orifice.

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13. A liquid chromatography system, comprising:

a first substrate having a first surface and a second surface, the first substrate defining an entrance opening on the first surface, a fluid reservoir recessed from the second surface, a first channel extending between the entrance opening and the reservoir, a second channel recessed from the second surface, and a plurality of posts extending from the second channel; and

a cover substrate bonded to the first substrate to enclose the reservoir and the second channel adjacent the cover substrate,

wherein the first and/or the cover substrate defines an exit and wherein the second channel extends between the exit and the reservoir.

14. The liquid chromatography system of claim 13, further comprising an insulating layer provided over the surfaces of the separation channel and the plurality of posts.

15. The liquid chromatography system of claim 13, wherein the posts are spaced apart from each other by no more than 5  $\mu\text{m}$ .

16. The liquid chromatography system of claim 13, wherein the first substrate defines a plurality of entrance openings on the first surface, a plurality of reservoirs recessed from the second surface each corresponding to one of the plurality of entrance openings, one or more first channels each corresponding to and extending between one of the plurality of entrance openings and the corresponding reservoir, and a plurality of second channels recessed from the second surface, wherein the first and/or the cover substrate defines a plurality of exits each corresponding to one of the plurality of reservoirs, and wherein each second channel corresponds to and extends between one of the plurality of reservoirs and the corresponding exit.

[illegible]

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a microfabricated device defining a liquid chromatography device comprising an entrance for receiving an analyte, the device further defining an electrospray device including a nozzle, the electrospray device being configured to receive the analyte from the liquid chromatography device and to generate an electrospray; and a mass spectrometer comprising a sampling orifice, said microfabricated device being positioned to eject the electrospray from the nozzle into the sampling orifice.

a second microfabricated structure defining an electrospray device including an entrance for receiving the analyte from the liquid chromatography device and a nozzle in fluid communication with the entrance, the electrospray device to generate an electrospray and wherein the electrospray nozzle is adapted to eject the electrospray from the nozzle into a sampling orifice of a mass spectrometer.



## **ABSTRACT**

An electrospray device, a liquid chromatography device and an electrospray-liquid chromatography system are disclosed. The electrospray device comprises a substrate defining a channel between an entrance orifice on an injection surface and an exit orifice on an ejection surface, a nozzle defined by a portion recessed from the ejection surface surrounding the exit orifice, and an electrode for application of an electric potential to the substrate to optimize and generate an electrospray; and, optionally, additional electrode(s) to further modify the electrospray. The liquid chromatography device comprises a separation substrate defining an introduction channel between an entrance orifice and a reservoir and a separation channel between the reservoir and an exit orifice, the separation channel being populated with separation posts perpendicular to the fluid flow; a cover substrate bonded to the separation substrate to enclose the reservoir and the separation channel adjacent the cover substrate; and, optionally, electrode(s) for application of a electric potential to the fluid. The exit orifice of the liquid chromatography device may be homogeneously interfaced with the entrance orifice of the electrospray device to form an integrated single system. An array of multiple systems may be fabricated in a single monolithic chip for rapid sequential fluid processing and generation of electrospray for subsequent analysis, such as by positioning the exit orifices of the electrospray devices near the sampling orifice of a mass spectrometer.

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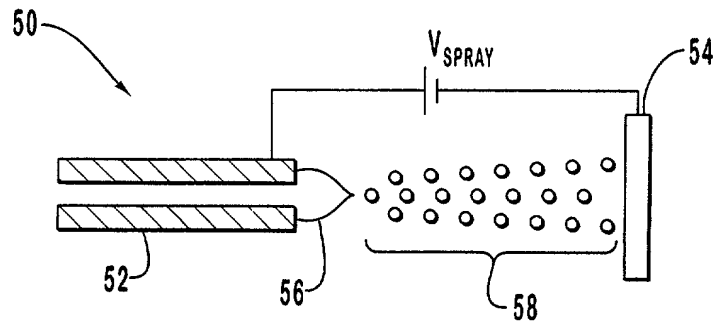


FIG. 1

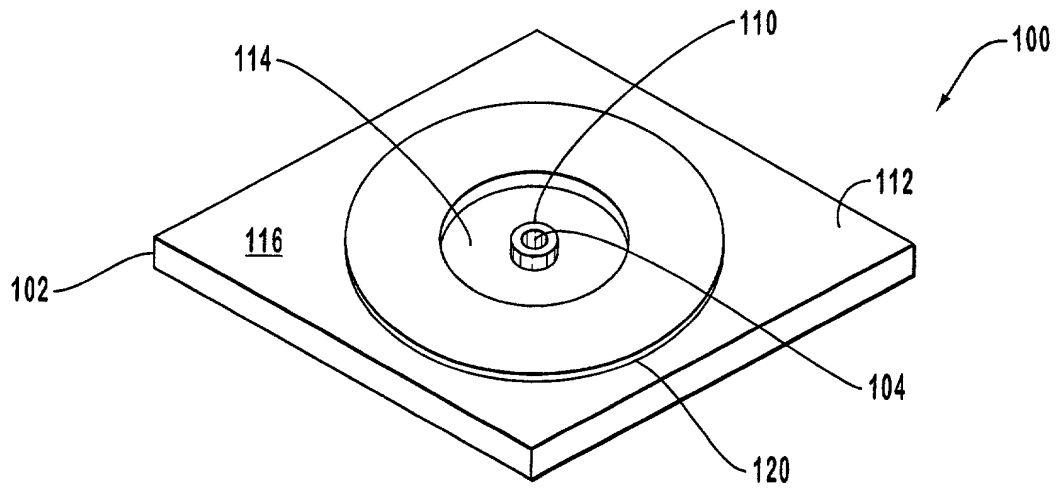


FIG. 2

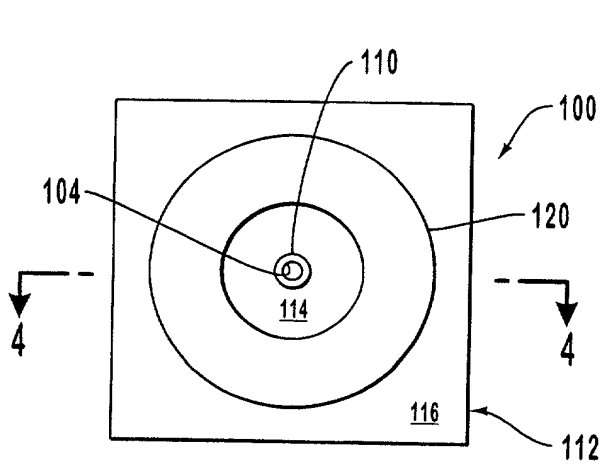


FIG. 3

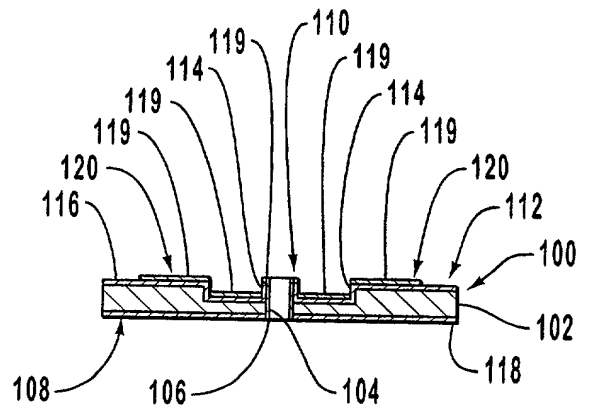


FIG. 4

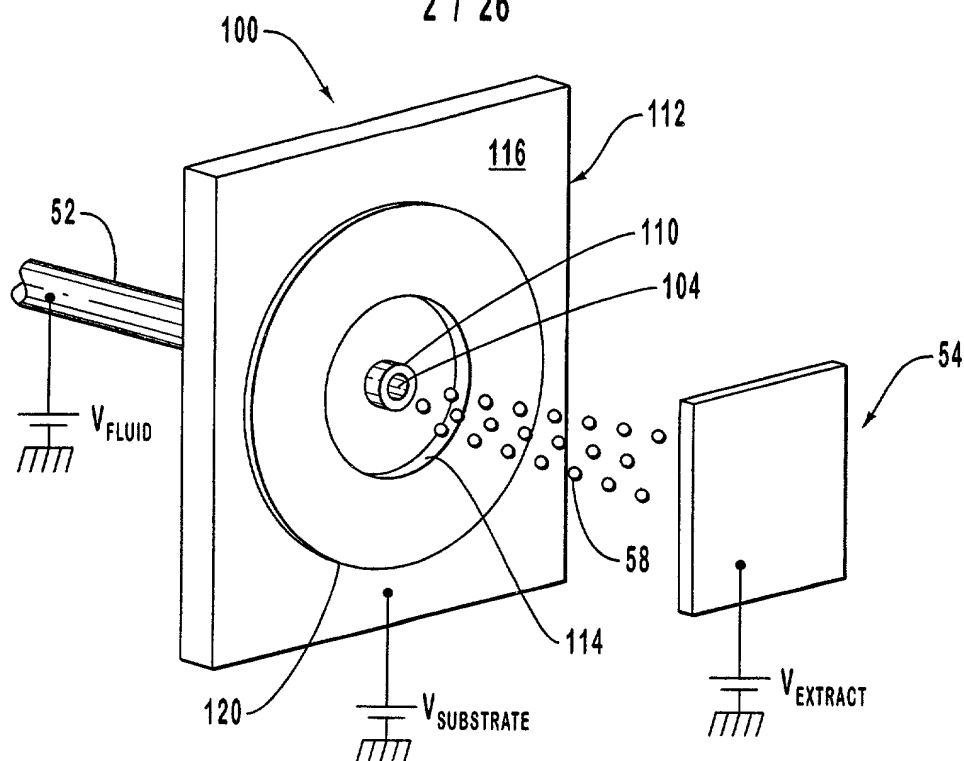


FIG. 5

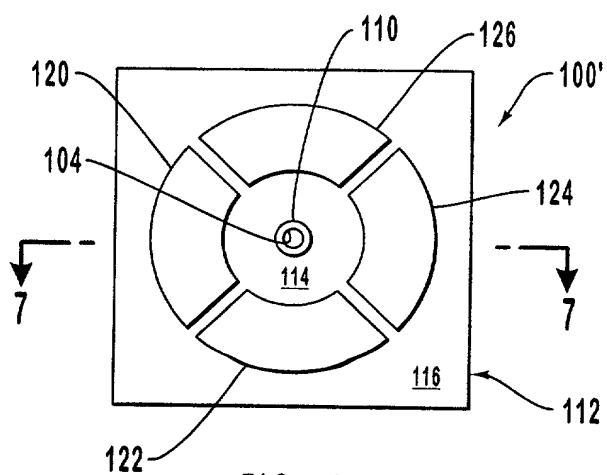
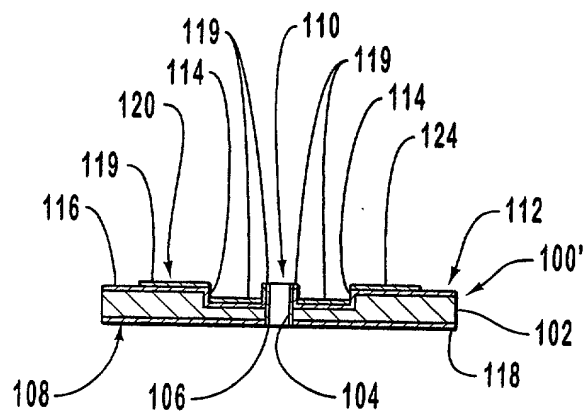


FIG. 6



710 7

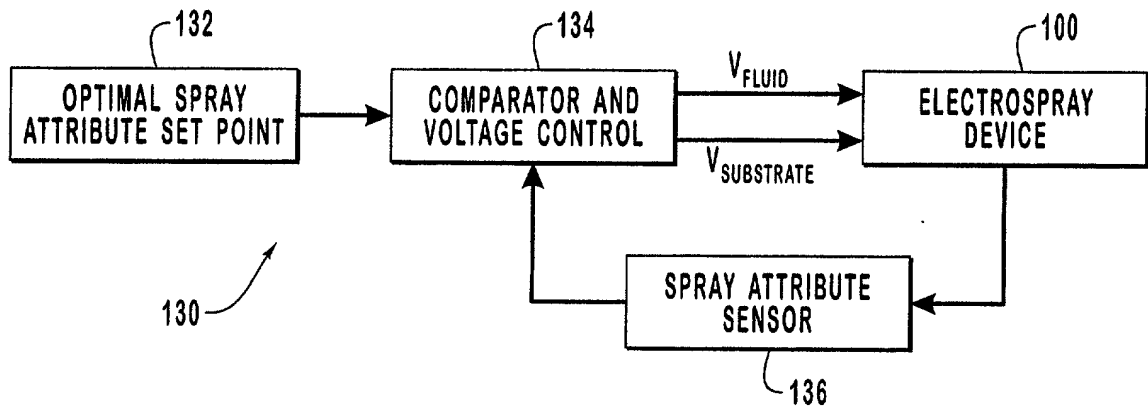


FIG. 8

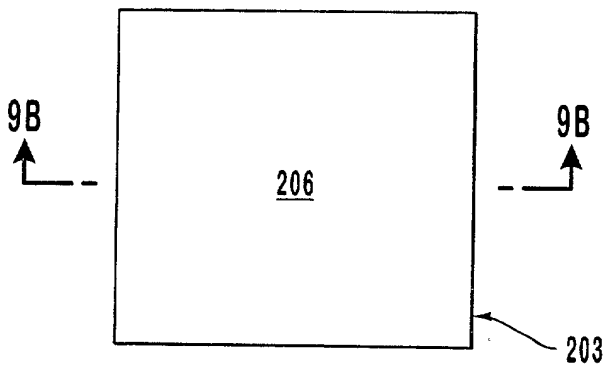


FIG. 9A

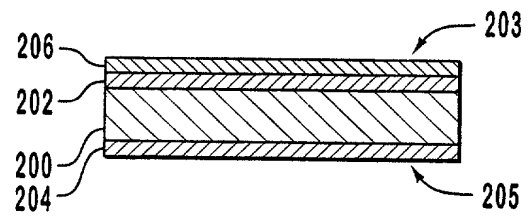
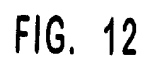
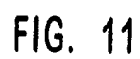
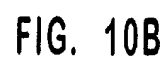
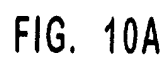


FIG. 9B



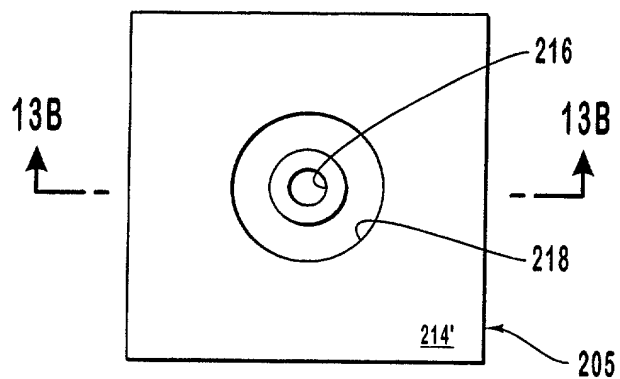


FIG. 13A

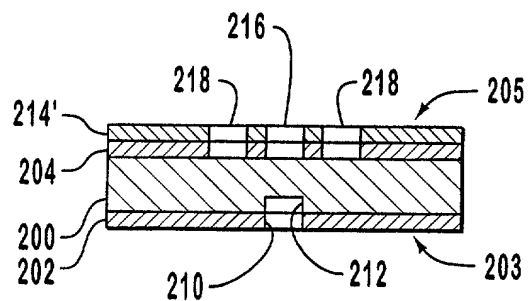


FIG. 13B

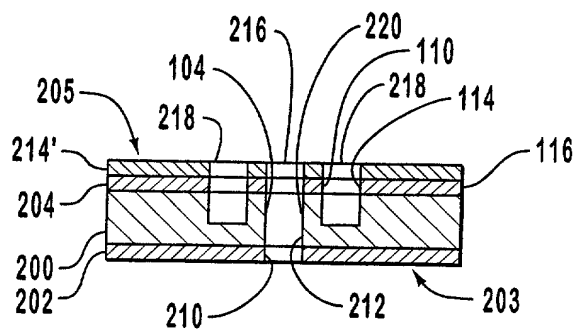


FIG. 14

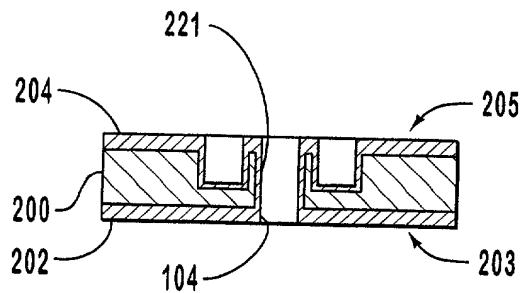


FIG. 15

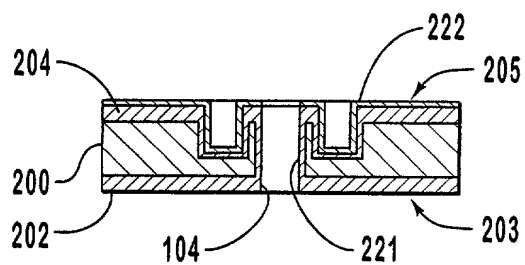


FIG. 16

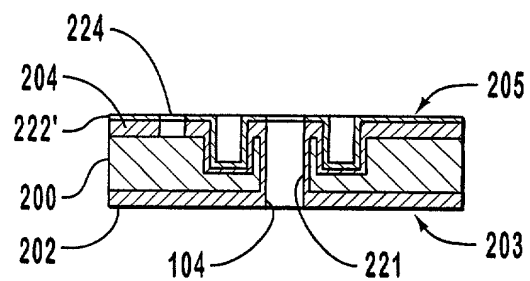


FIG. 17

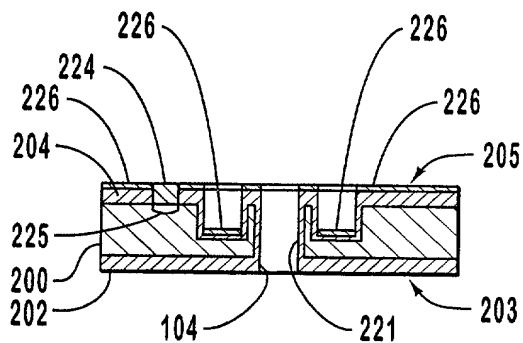


FIG. 18

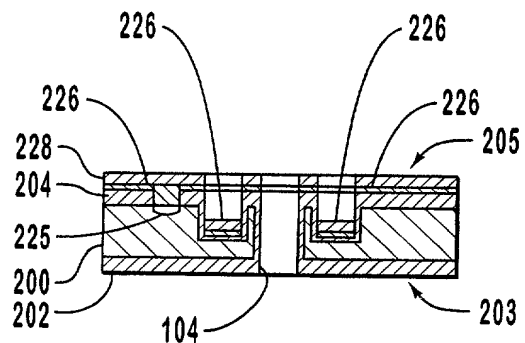


FIG. 19

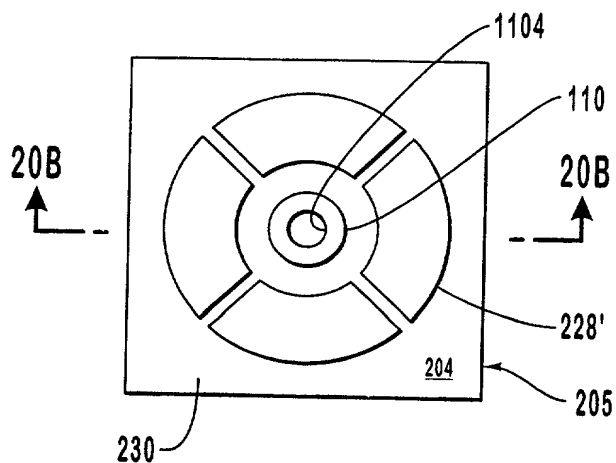


FIG. 20A

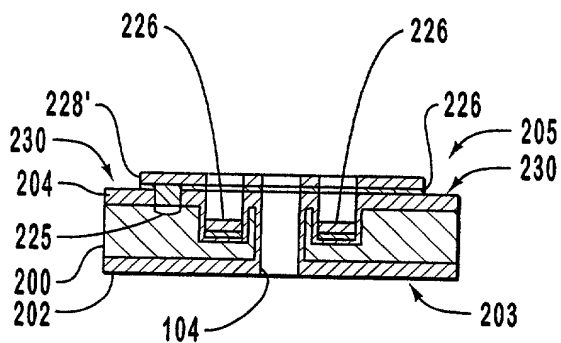


FIG. 20B

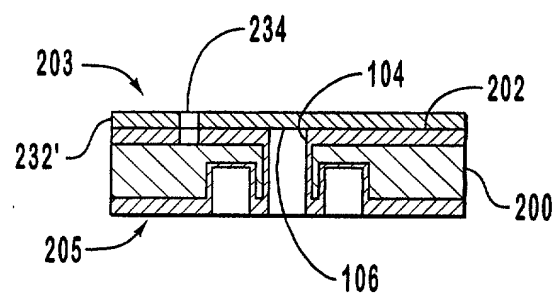


FIG. 20D

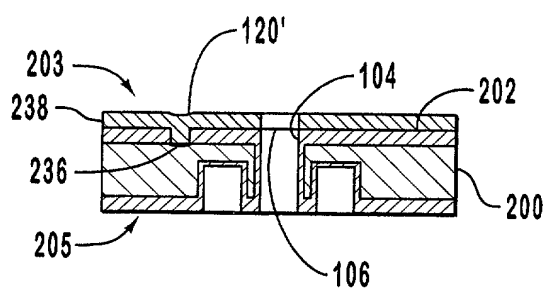


FIG. 20E

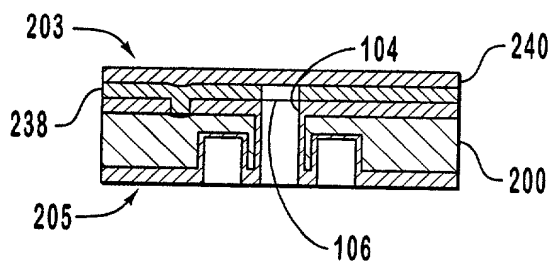


FIG. 20F

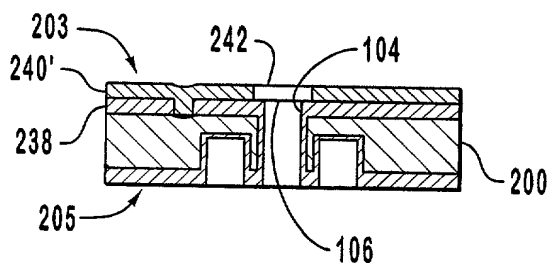


FIG. 20G



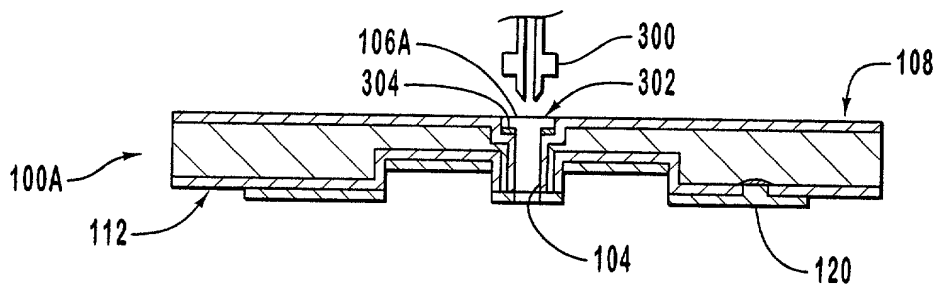


FIG. 21A

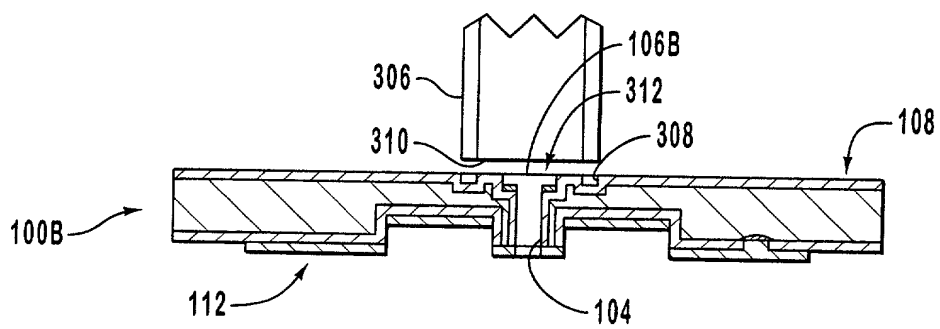


FIG. 21B

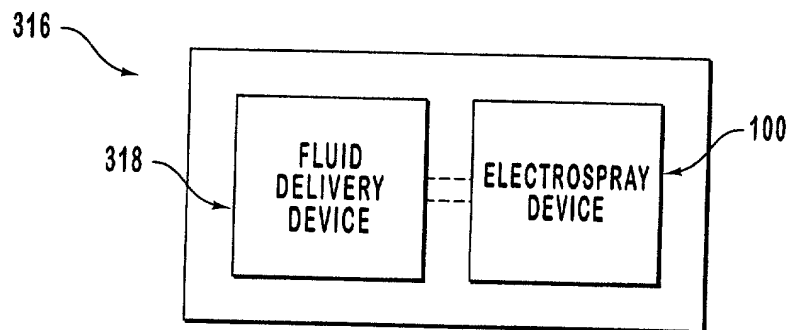


FIG. 22

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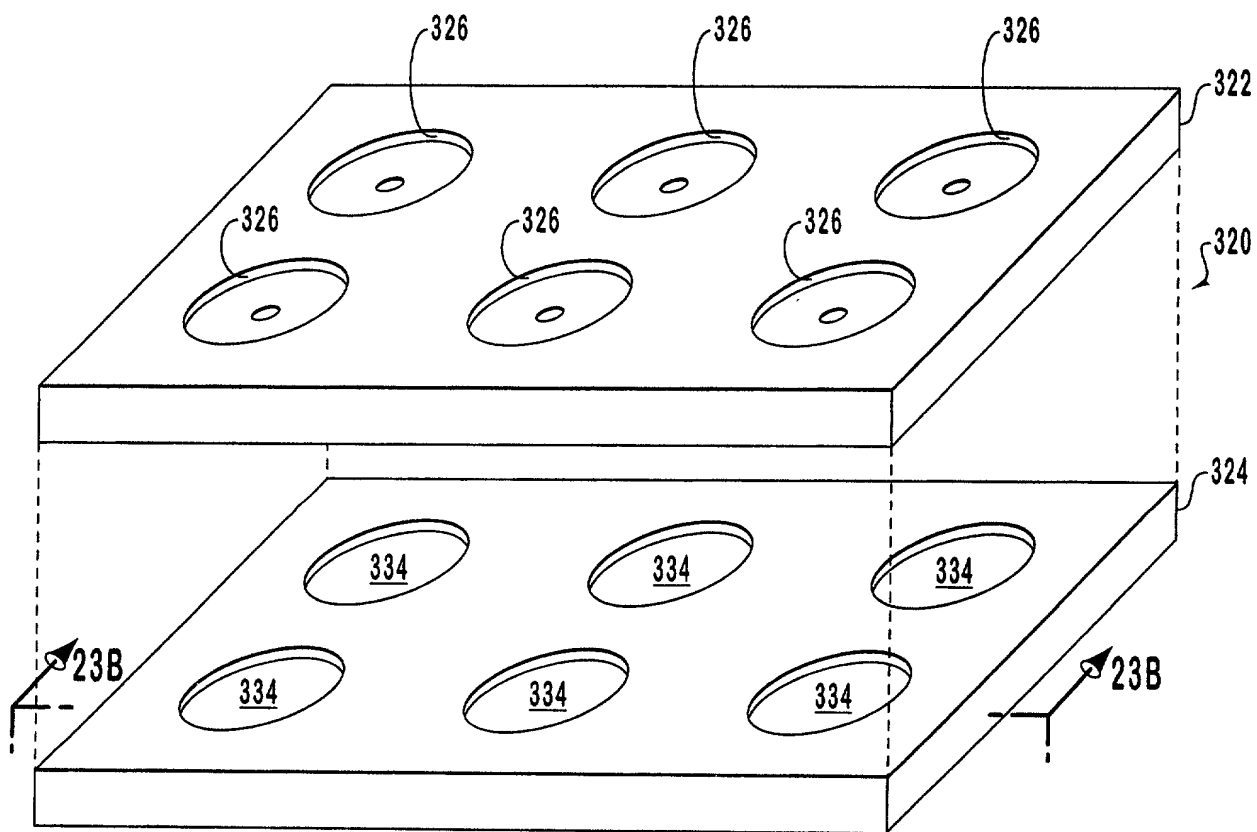


FIG. 23A

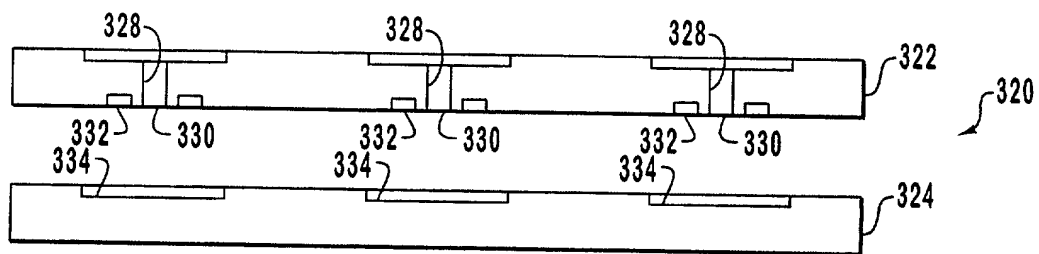


FIG. 23B

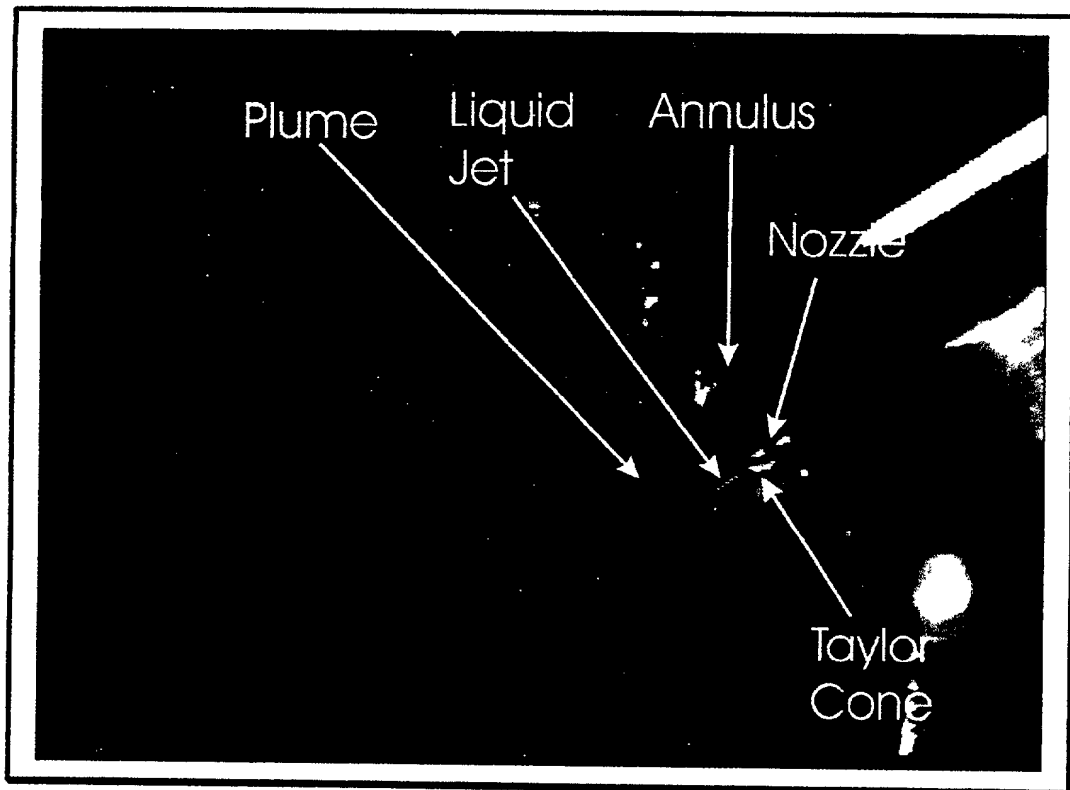


FIG. 24A

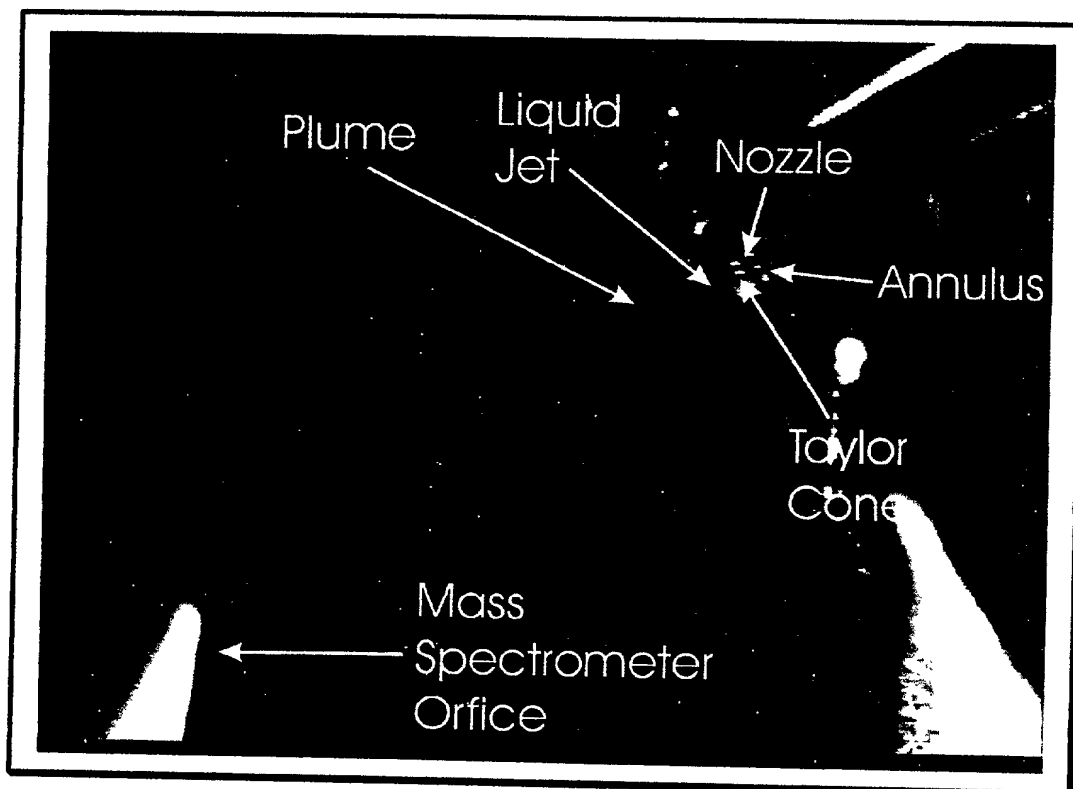
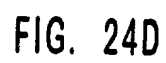
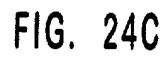


FIG. 24B

002207 6266960



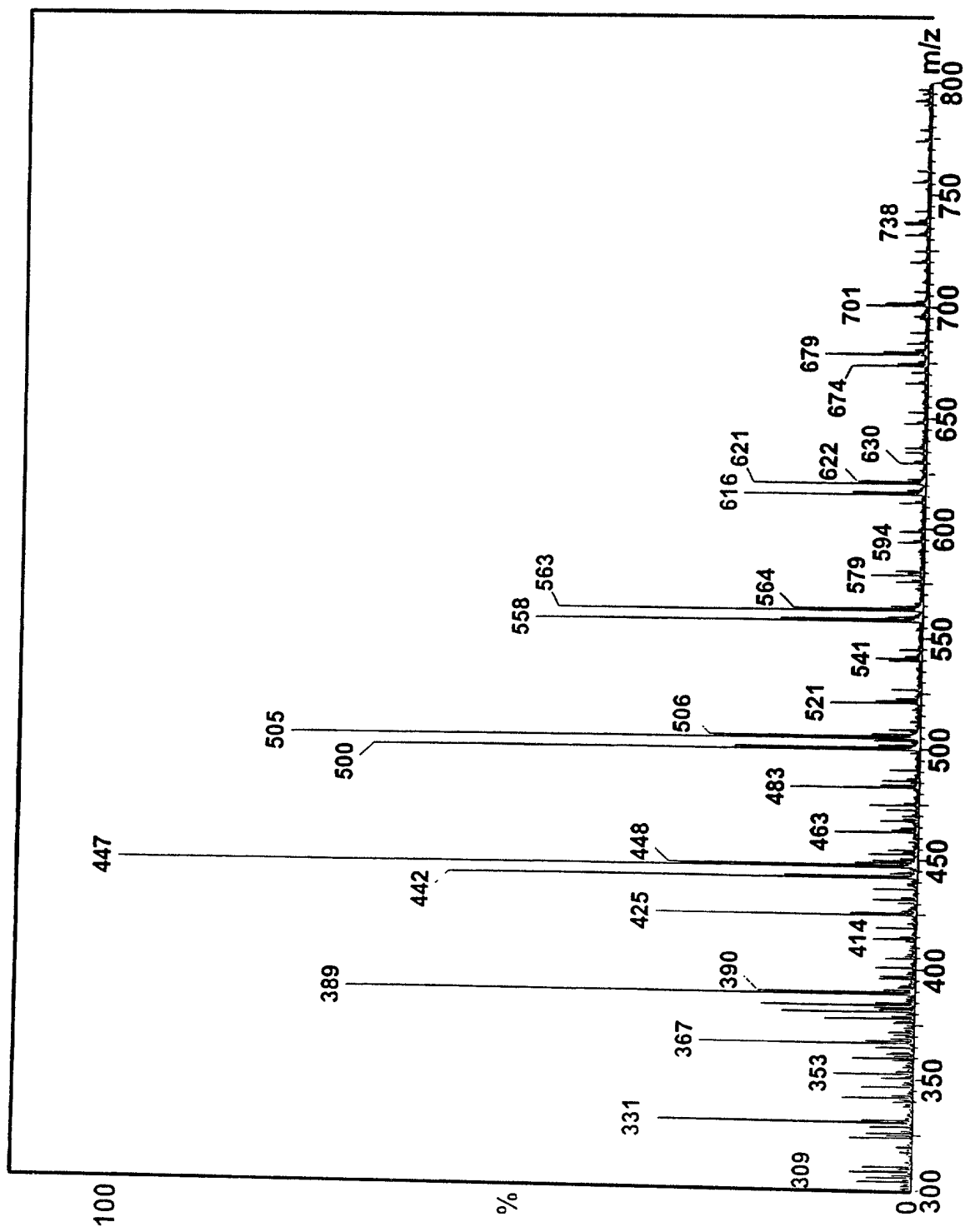


FIG. 24E

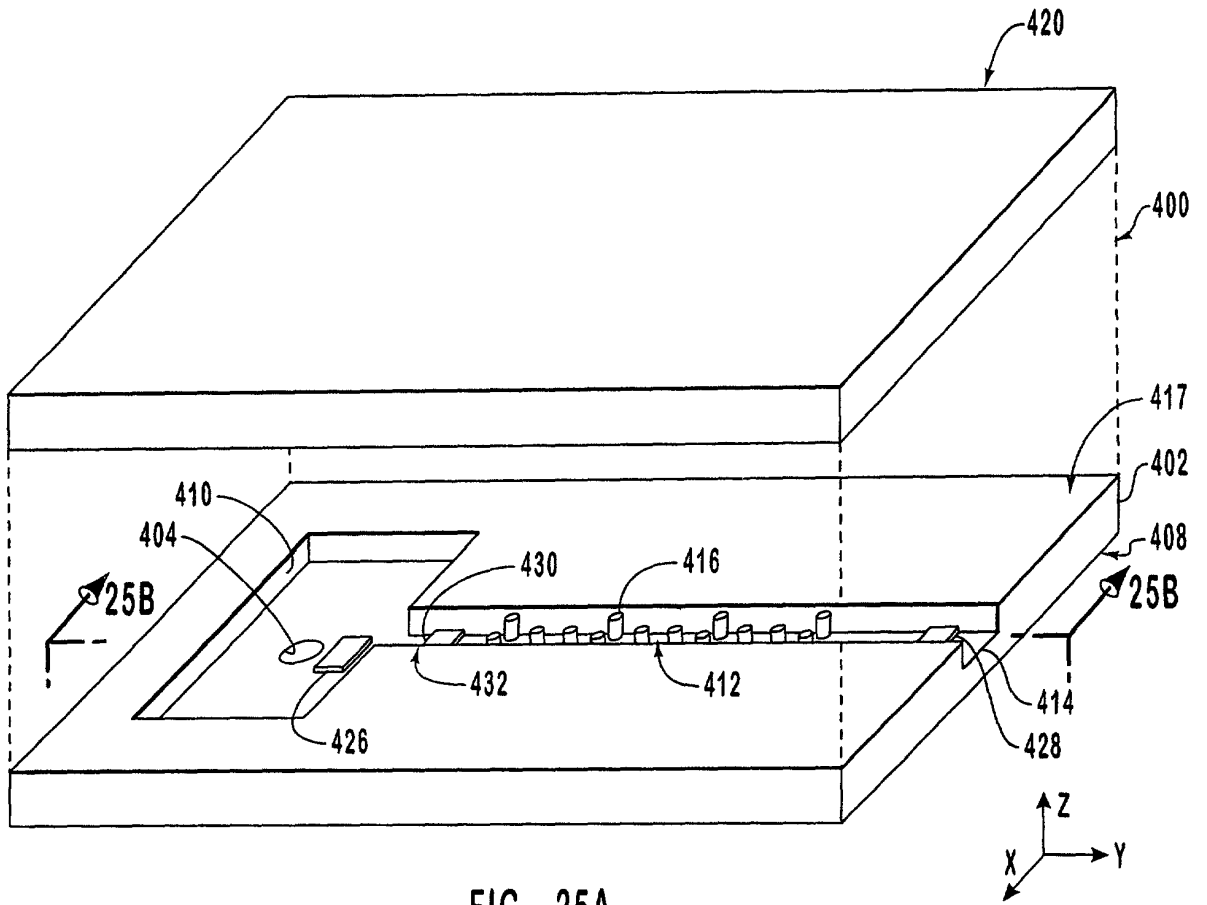


FIG. 25A

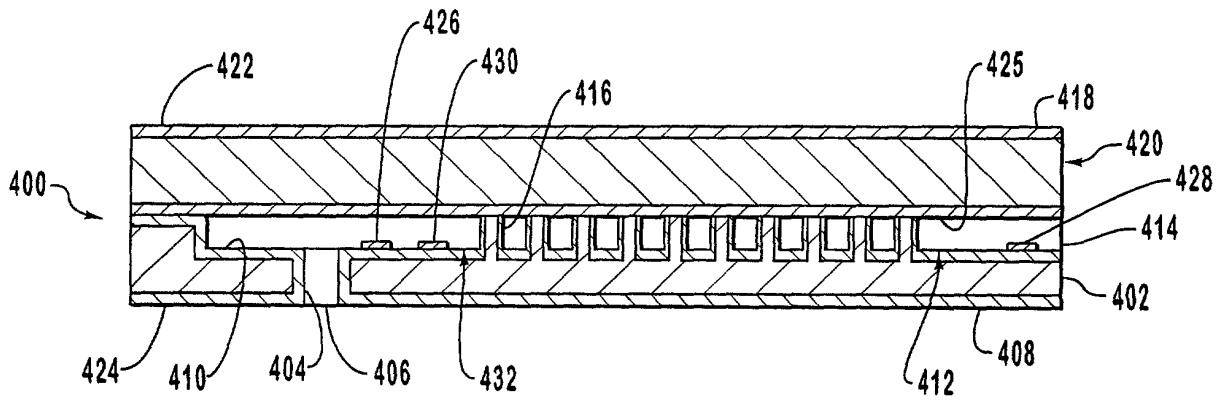


FIG. 25B

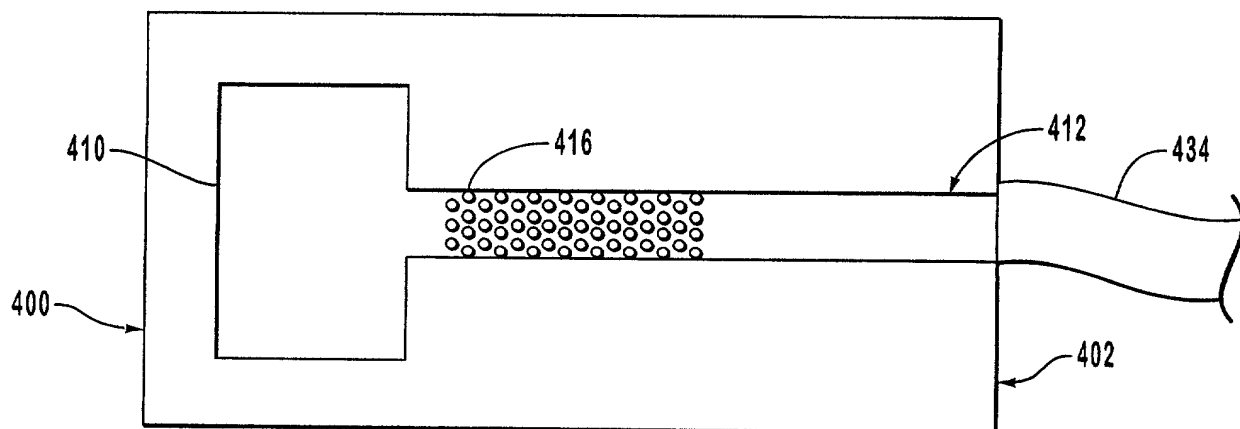


FIG. 26

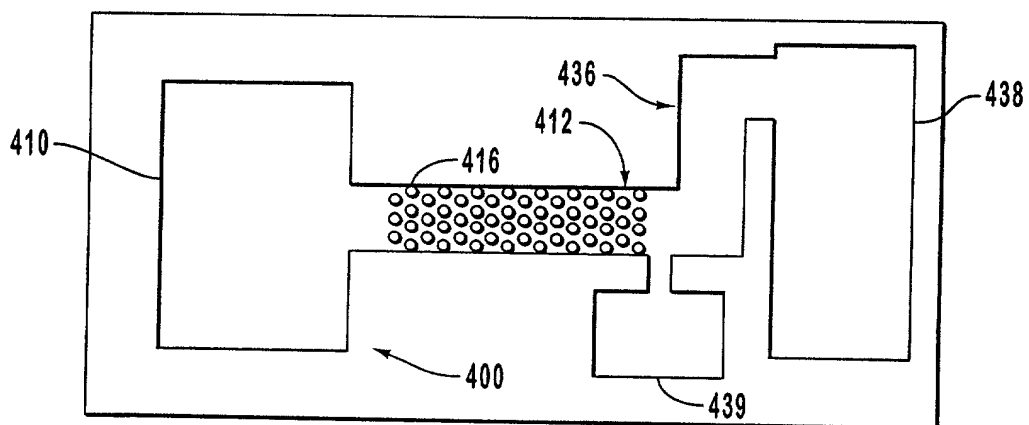


FIG. 27

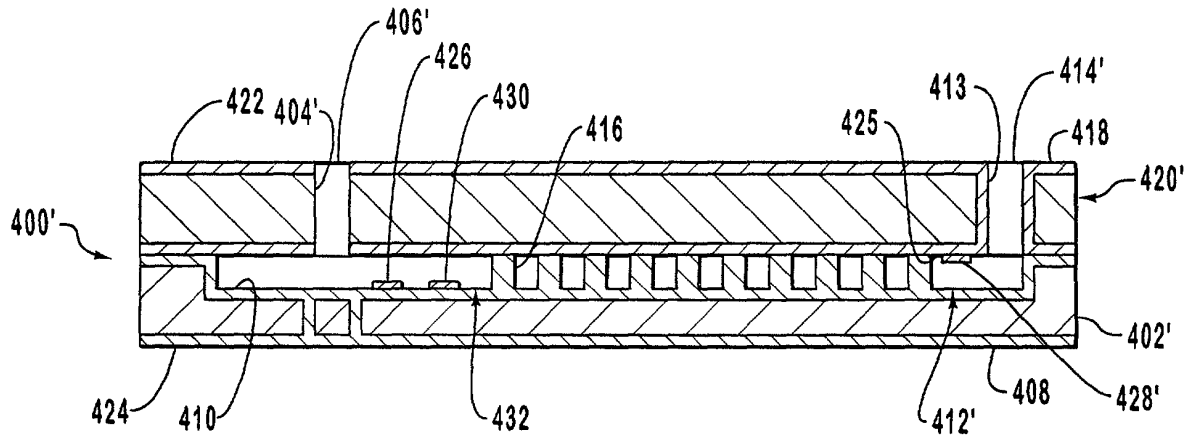


FIG. 28

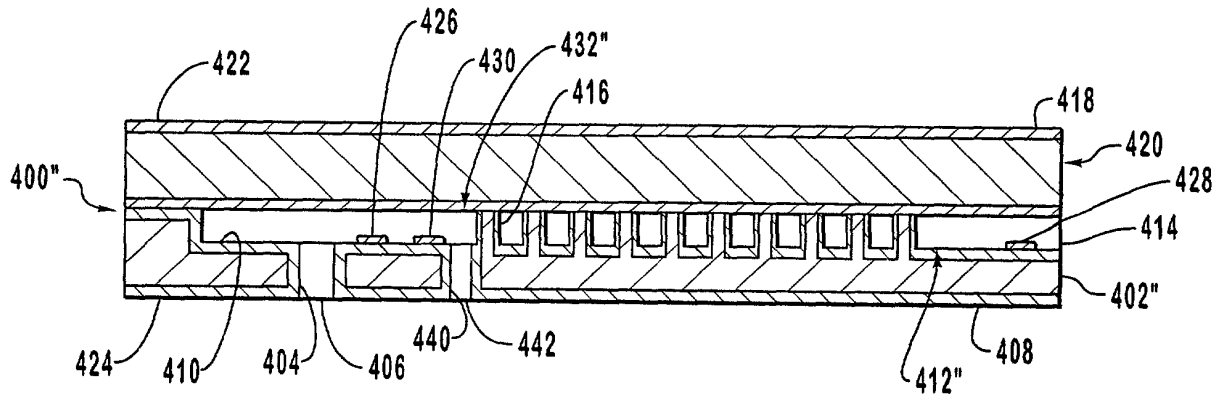


FIG. 29



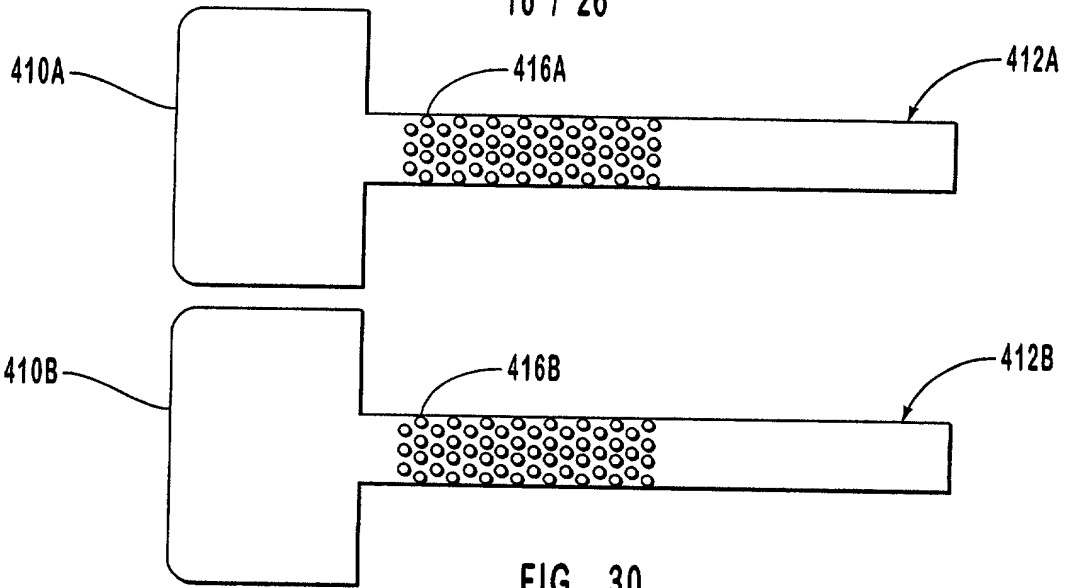


FIG. 30

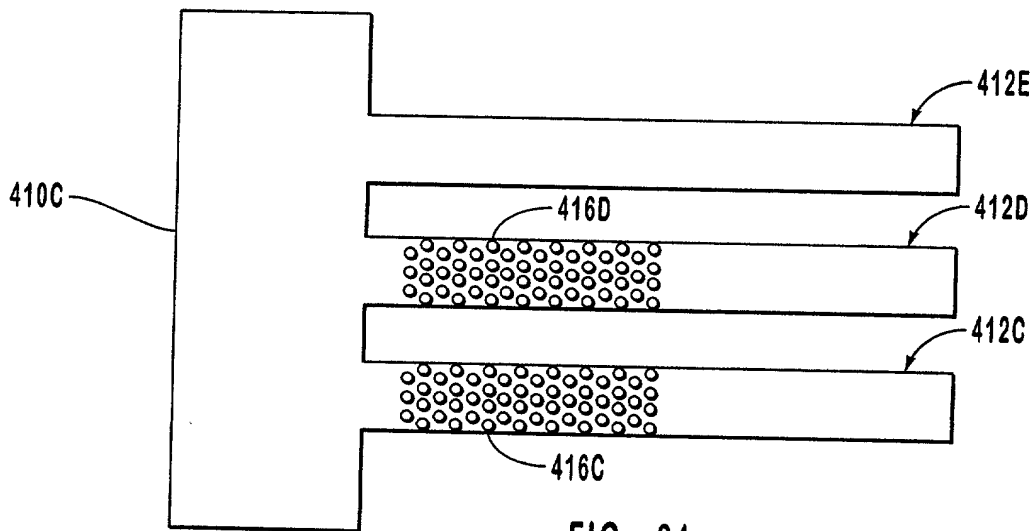
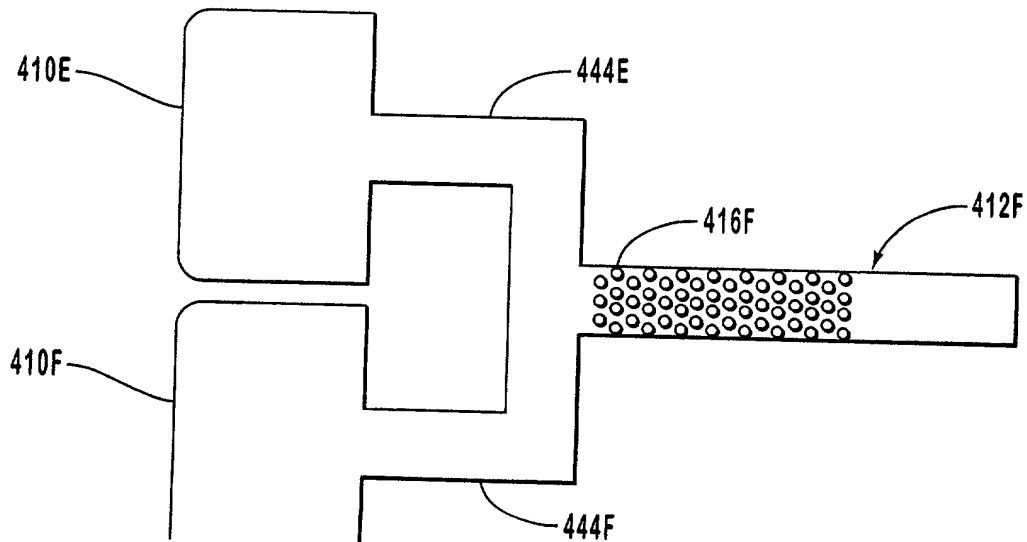
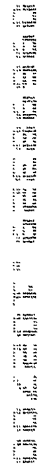
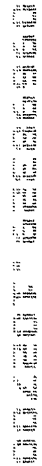


FIG. 31



[illegible][illegible]

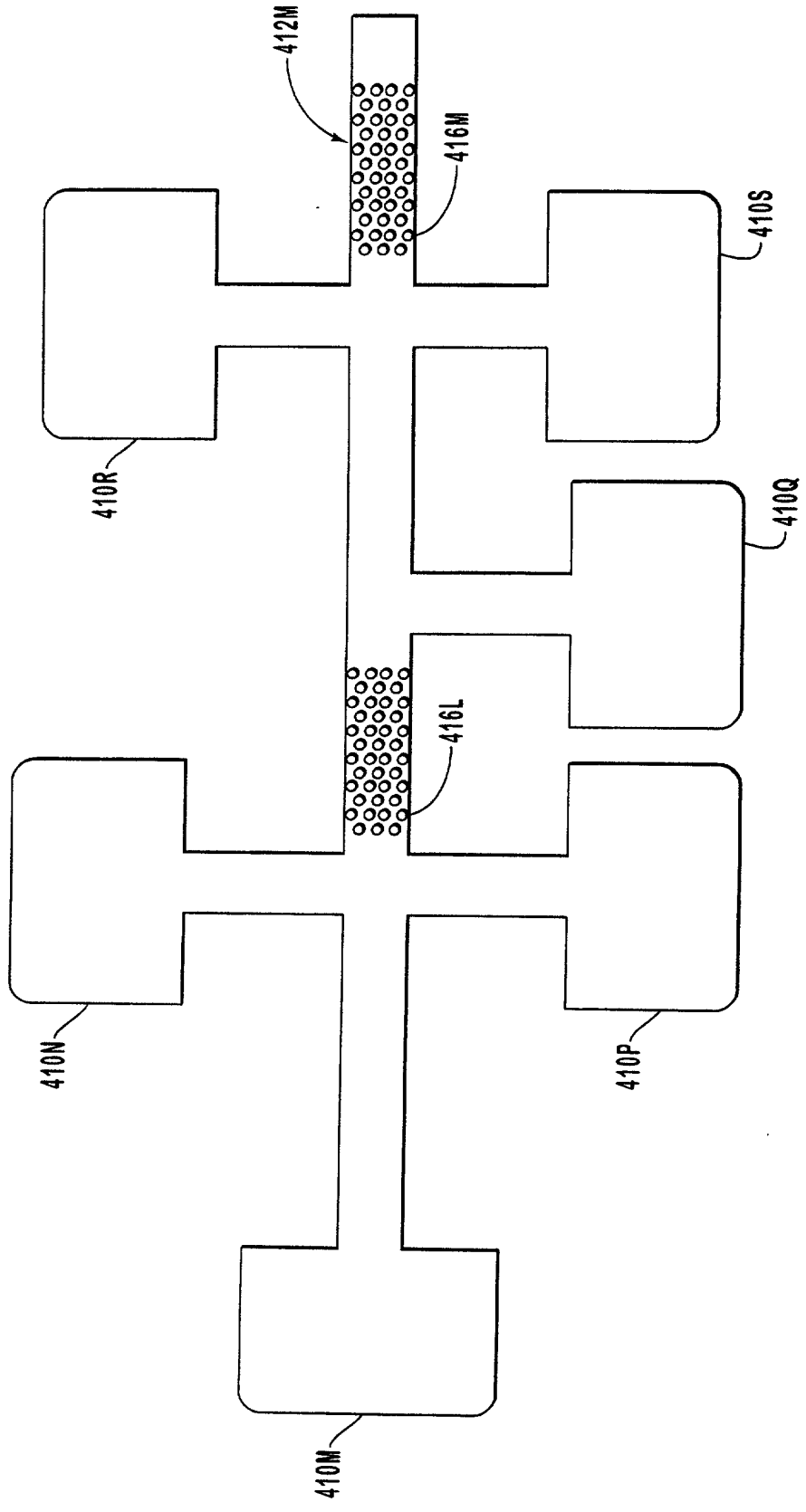


FIG. 35

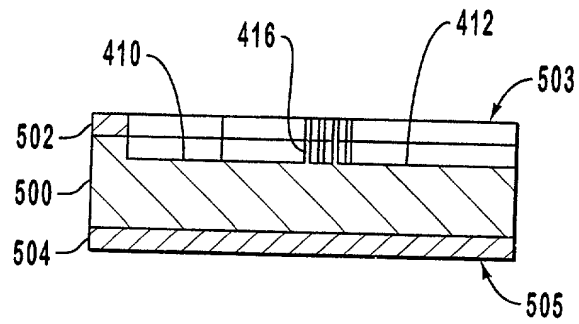
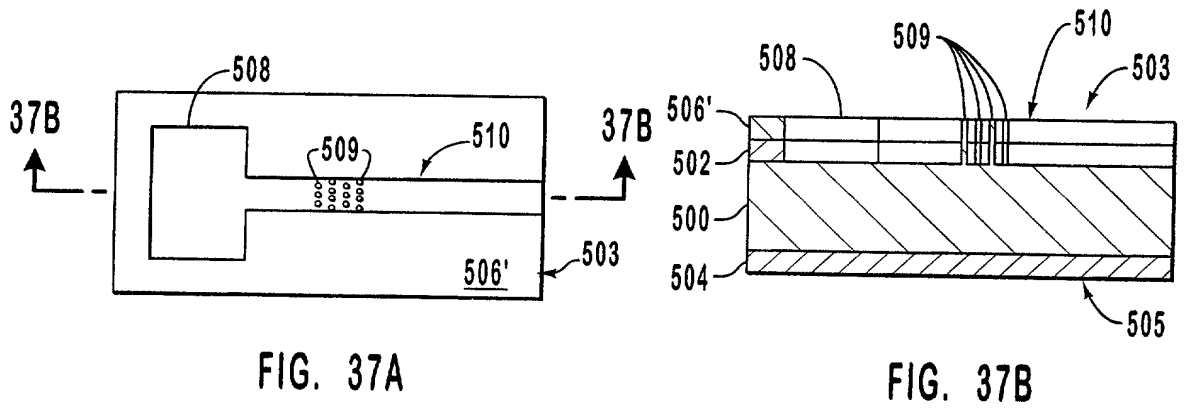
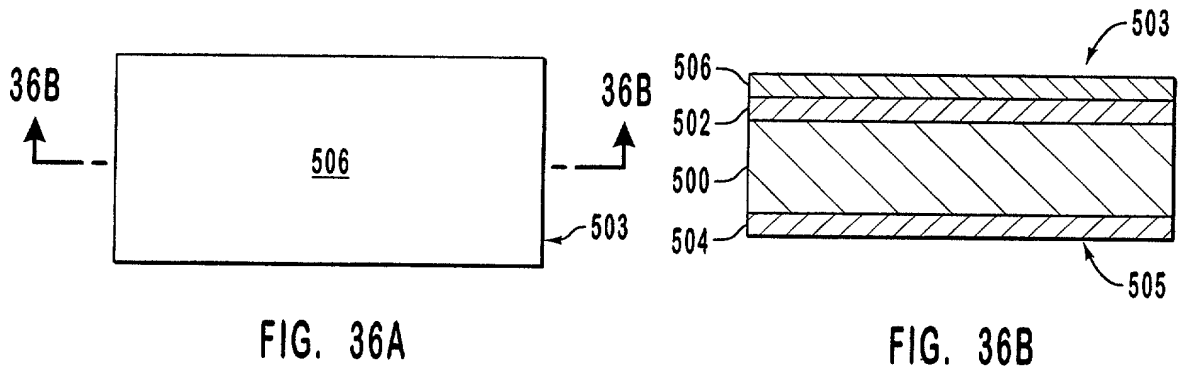


FIG. 38

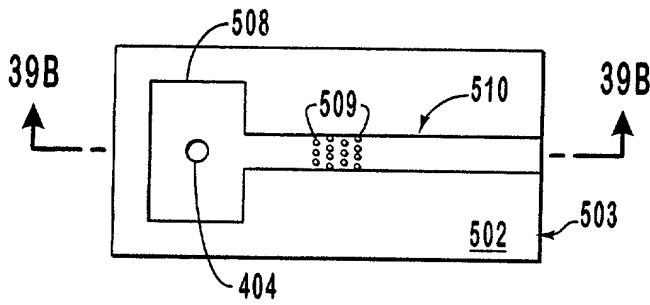


FIG. 39A

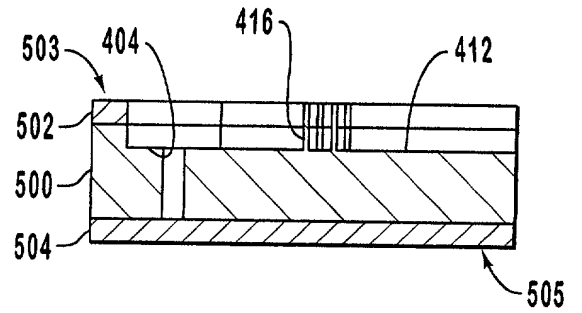


FIG. 39B

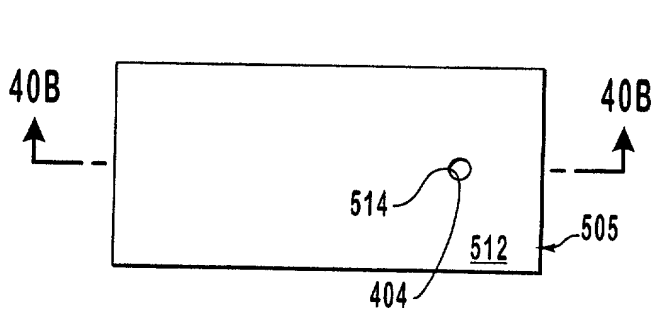


FIG. 40A

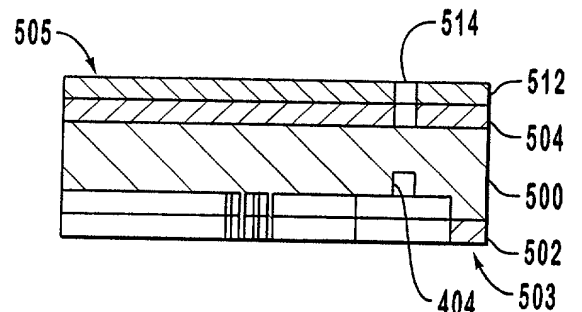


FIG. 40B

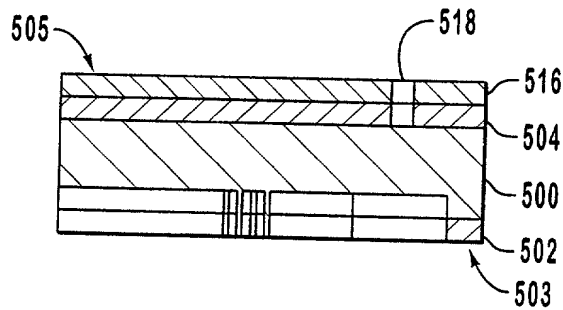


FIG. 41

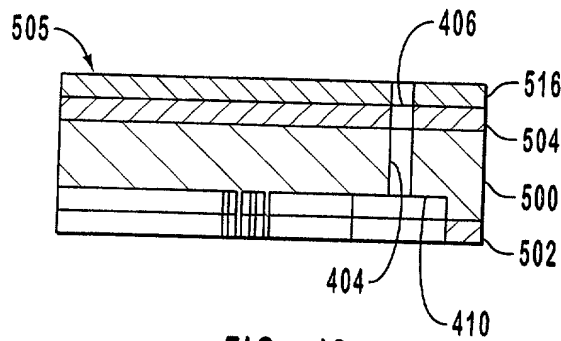


FIG. 42

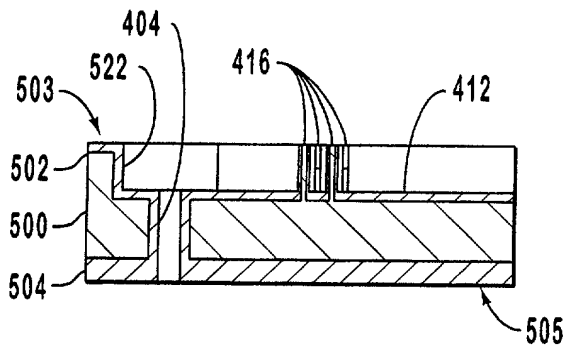


FIG. 43

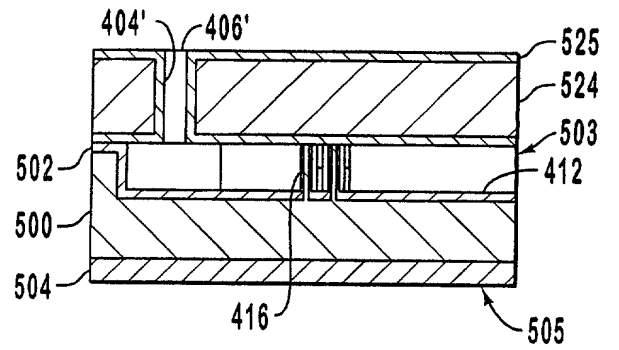


FIG. 45

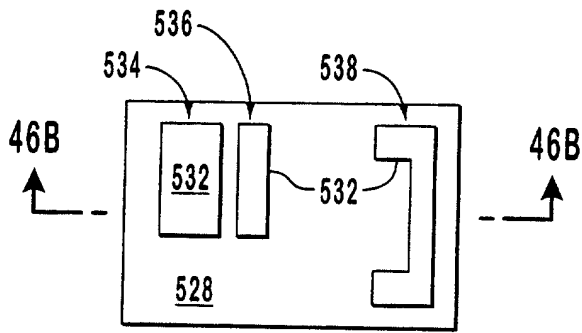


FIG. 46A

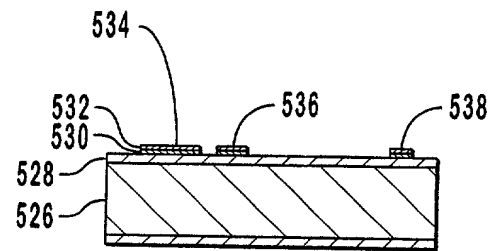


FIG. 46B

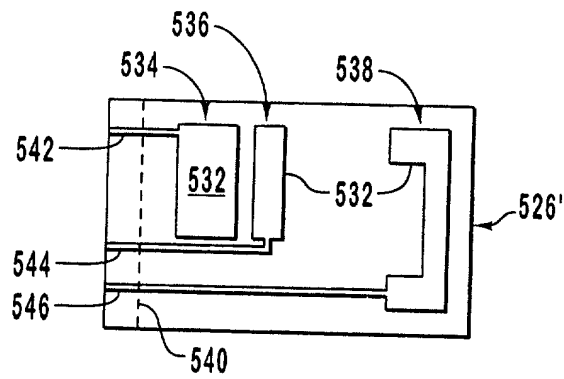


FIG. 46C



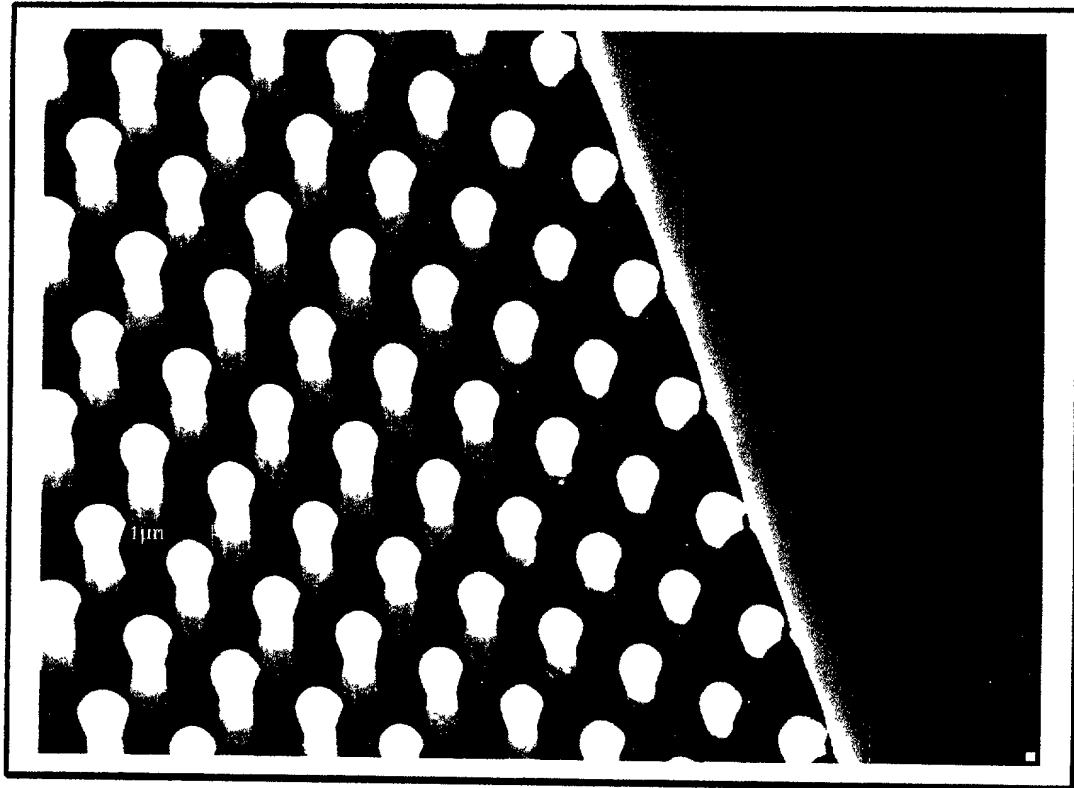


FIG. 44B

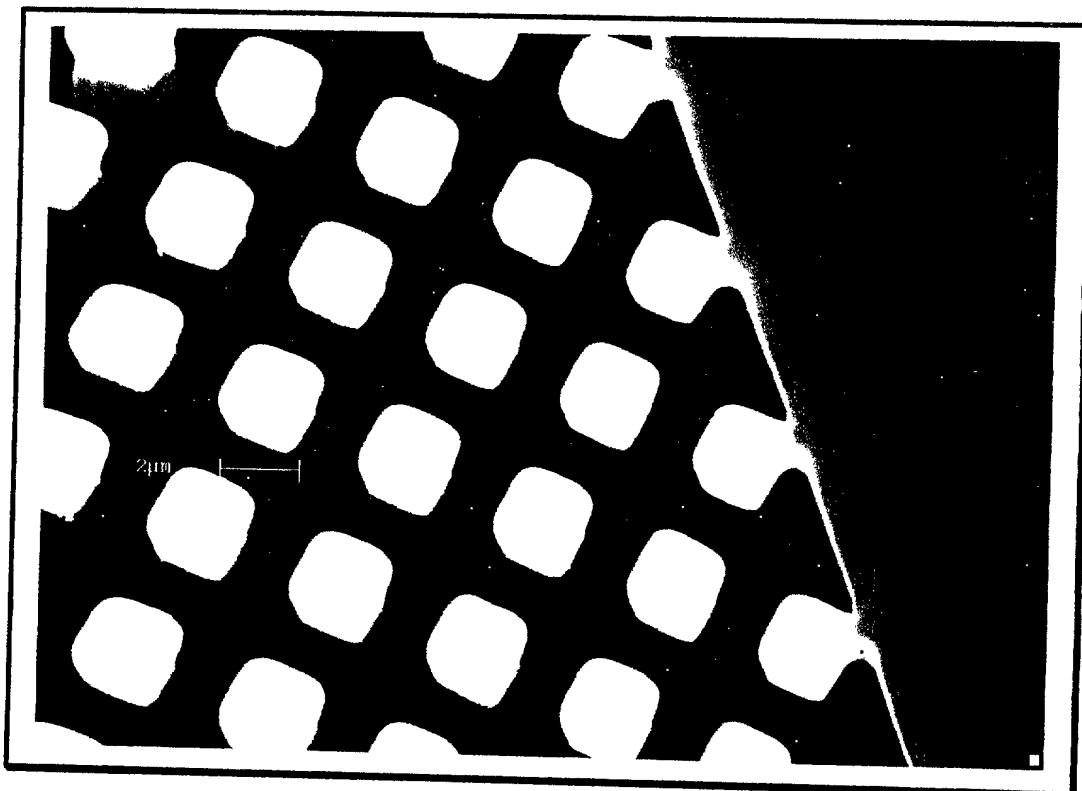


FIG. 44C

002007 00000000



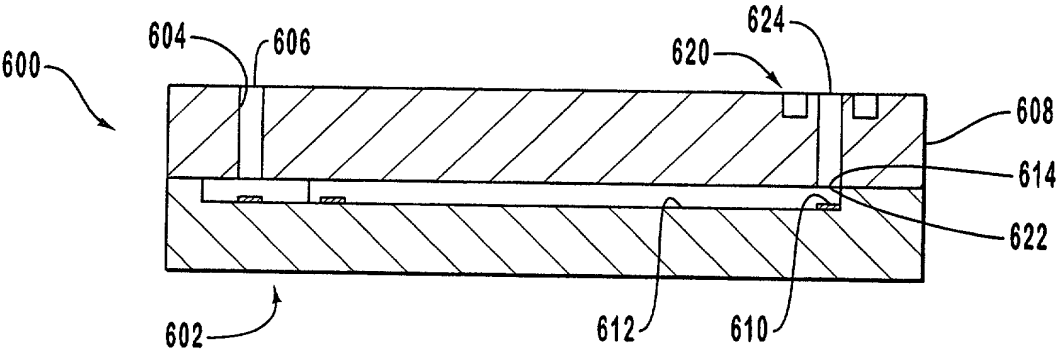


FIG. 47

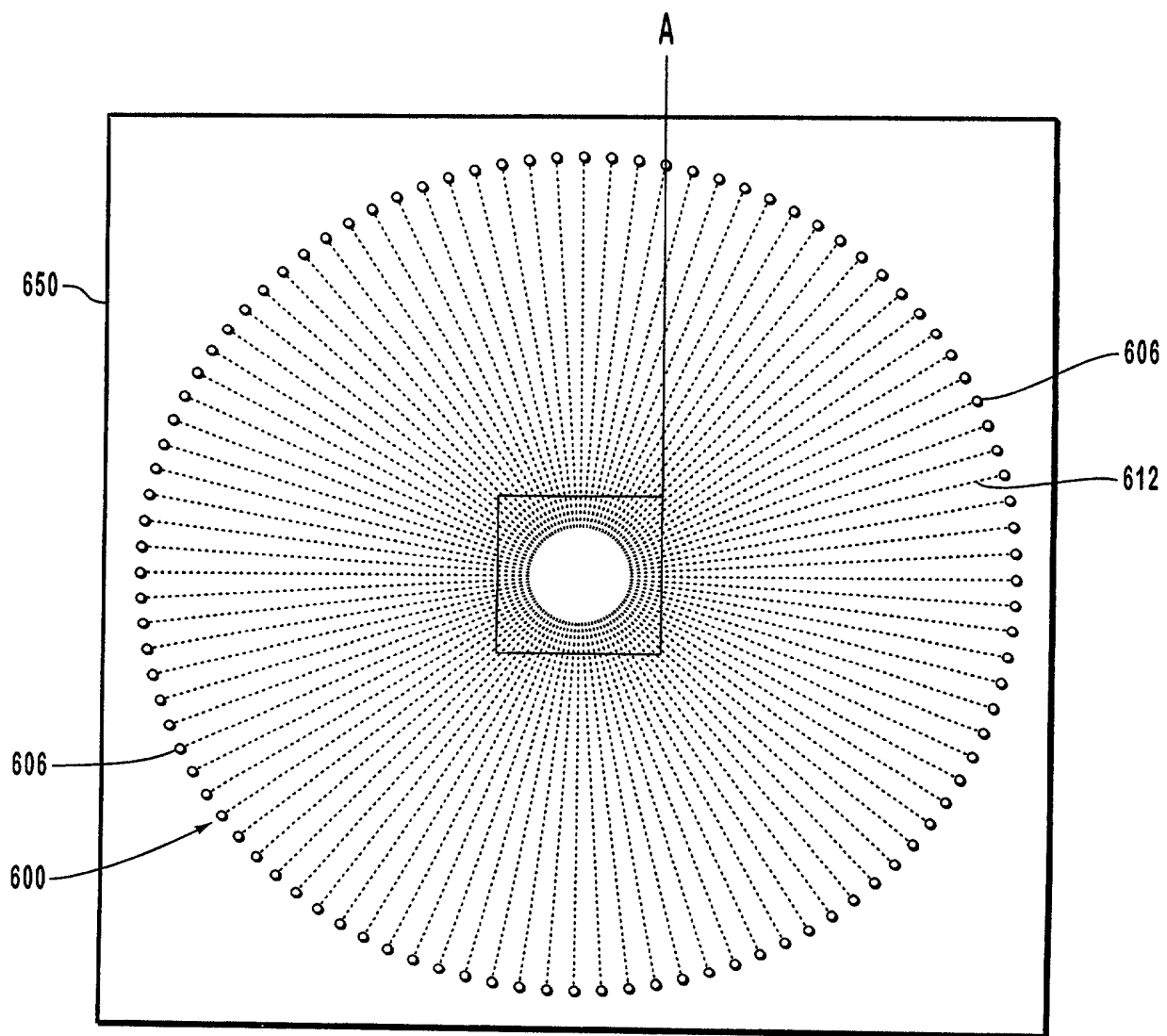


FIG. 48

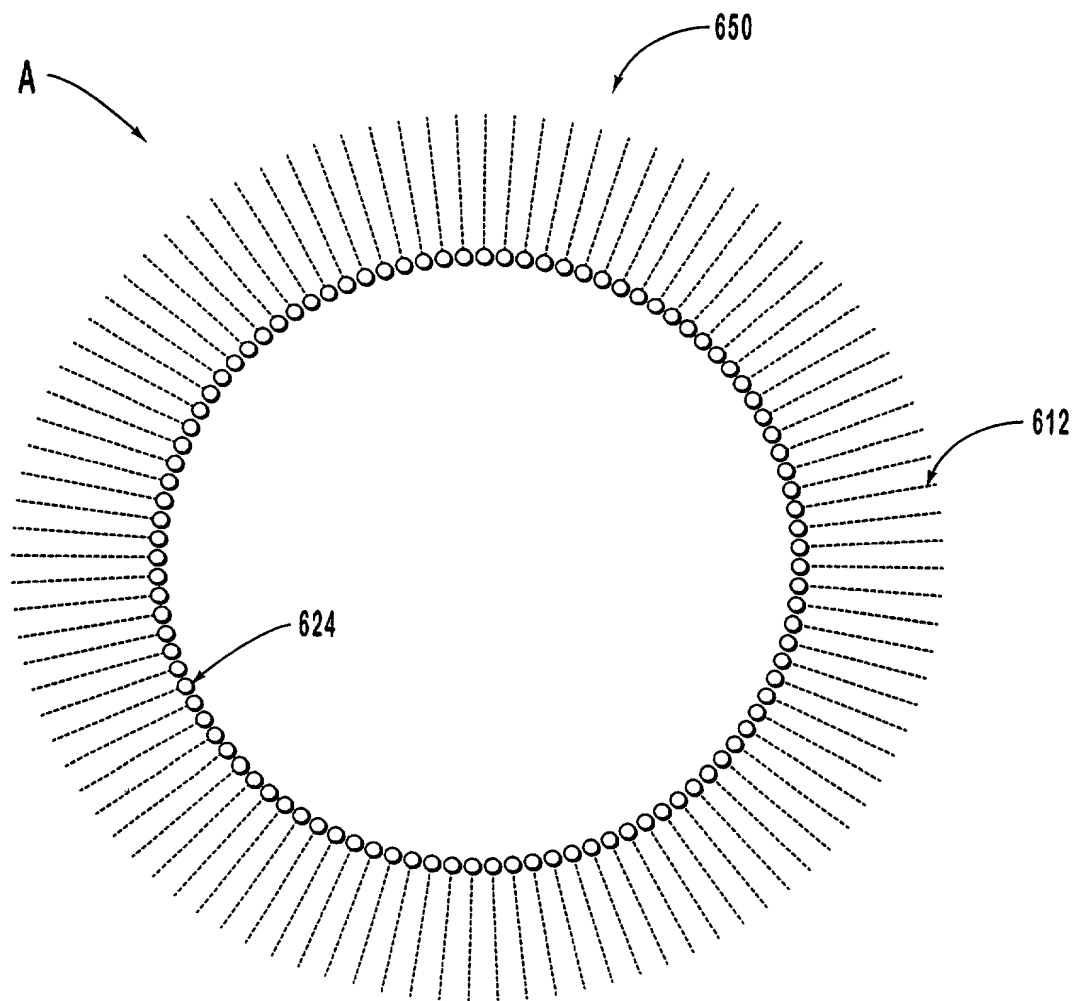


FIG. 49

00698339 102700 002201 62286560

COMBINED DECLARATION FOR PATENT  
APPLICATION AND POWER OF ATTORNEY  
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER

200701/1030

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID  
CHROMATOGRAPHY SYSTEM AND METHOD**

the specification of which (check only one item below):

☐ is attached hereto.

☒ was filed as U.S. Patent Application Serial No. 09/156,507 on September 17, 1998 and was amended on \_\_\_\_\_  
(if applicable).

☐ was filed as PCT International Application No. \_\_\_\_\_ on \_\_\_\_\_ and was amended under PCT Article 19 on  
(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specifications, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:**

COUNTRY (IF PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application:

**PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:**

U.S. APPLICATIONS		STATUS (Check One)			
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

ATTORNEY'S DOCKET NUMBER  
200701/1030

Send Correspondence to: **Michael L. Goldman**  
**Nixon, Hargrave, Devans & Doyle LLP**  
**Clinton Square, P.O. Box 1051**  
**Rochester, New York 14603**

201	FULL NAME OF INVENTOR	FAMILY NAME Moon	FIRST GIVEN NAME James	SECOND GIVEN NAME E.
	RESIDENCE & CITIZENSHIP	CITY Ithaca	STATE/FOREIGN COUNTRY New York	COUNTRY OF CITIZENSHIP United States
	POST OFFICE ADDRESS	P.O. ADDRESS 122 E. Remington Road	CITY Ithaca	STATE & ZIP CODE/CTRY New York 14850/U.S.A./
202	FULL NAME OF INVENTOR	FAMILY NAME Davis	FIRST GIVEN NAME Timothy	SECOND GIVEN NAME J.
	RESIDENCE & CITIZENSHIP	CITY Trumansburg	STATE/FOREIGN COUNTRY New York	COUNTRY OF CITIZENSHIP United States
	POST OFFICE ADDRESS	P.O. ADDRESS 2283 State Route 96	CITY Trumansburg	STATE & ZIP CODE/CTRY New York 14886/U.S.A.
203	FULL NAME OF INVENTOR	FAMILY NAME Galvin	FIRST GIVEN NAME Gregory	SECOND GIVEN NAME J.
	RESIDENCE & CITIZENSHIP	CITY Ithaca	STATE/FOREIGN COUNTRY New York	COUNTRY OF CITIZENSHIP United States
	POST OFFICE ADDRESS	P.O. ADDRESS 124 E. King Road	CITY Ithaca	STATE & ZIP CODE/CTRY New York 14850/U.S.A.
204	FULL NAME OF INVENTOR	FAMILY NAME Schultz	FIRST GIVEN NAME Gary	SECOND GIVEN NAME A.
	RESIDENCE & CITIZENSHIP	CITY Ithaca	STATE/FOREIGN COUNTRY New York	COUNTRY OF CITIZENSHIP United States
	POST OFFICE ADDRESS	P.O. ADDRESS 323 Richard Place	CITY Ithaca	STATE & ZIP CODE/CTRY New York 14850/U.S.A.
205	FULL NAME OF INVENTOR	FAMILY NAME Corso	FIRST GIVEN NAME Thomas	SECOND GIVEN NAME N.
	RESIDENCE & CITIZENSHIP	CITY Freeville	STATE/FOREIGN COUNTRY New York	COUNTRY OF CITIZENSHIP United States
	POST OFFICE ADDRESS	P.O. ADDRESS 5 Mineah Road, Apt. 7	CITY Freeville	STATE & ZIP CODE/CTRY New York 13068/U.S.A.
206	FULL NAME OF INVENTOR	FAMILY NAME Lowes	FIRST GIVEN NAME Stephen	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY Freeville	STATE/FOREIGN COUNTRY New York	COUNTRY OF CITIZENSHIP United States
	POST OFFICE ADDRESS	P.O. ADDRESS 418 Ferguson Road	CITY Freeville	STATE & ZIP CODE/CTRY New York 13068/U.S.A.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
DATE	DATE	DATE
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE 12 MAR 99	DATE 12 MAR 99	DATE 12 MAR 99

Page 3 of 3

COMBINED DECLARATION FOR PATENT  
APPLICATION AND POWER OF ATTORNEY  
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER

200701/1030

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID  
CHROMATOGRAPHY SYSTEM AND METHOD**

the specification of which (check only one item below):

- ☐ is attached hereto.
- ☒ was filed as U.S. Patent Application Serial No. 09/156,507 on September 17, 1998 and was amended on \_\_\_\_\_ (if applicable).
- ☐ was filed as PCT International Application No. \_\_\_\_\_ on \_\_\_\_\_ and was amended under PCT Article 19 on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specifications, including the claims, as amended by any amendment referred to above.

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PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (IF PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check One)			
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

002007 0226560

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Continue)				ATTORNEY'S DOCKET NUMBER 200701/1030	
POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. Michael L. Goldman, Registration No. 30,727, Gunnar G. Leinberg, Registration No. 35,584; Dennis M. Connolly, Registration No. 40,964; Edwin V. Merkel, Registration No. 40,087, Jeffery B. Arnold, Registration No. 39,540					
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201	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201

DATE March 11 1999

DATE March 11, 1999

SIGNATURE OF INVENTOR 202

DATE Nov 11 199

DATE Mar 11, 1999

SIGNATURE OF INVENTOR 203

DATE 3/14/99

DATE 3/11/99

SIGNATURE OF INVENTOR 204

SIGNATURE OF INVENTOR 205

SIGNATURE OF INVENTOR 206

DATE \_\_\_\_\_

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Express Mail Label No. EL675131054US

PATENT APPLICATION

Docket: 14917.1.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: )  
Moon et al. )  
Prior Application )  
Serial No.: 09/156,507 ) Art Unit  
For: INTEGRATED MONOLITHIC MICROFABRICATED ) 1741  
ELECTROSPRAY AND LIQUID )  
CHROMATOGRAPHY SYSTEM AND METHOD )

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents  
Washington, D. C. 20231

Sir:

Please recognize KYLE H. FLINDT, Registration No. 42,539, and WILLIAM J. ATHAY, Registration No. 44,515 as associate attorneys for us in the above-entitled application. Please address all future written and telephonic communications to:

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Dated this 27th day of October 2000.

Respectfully submitted,



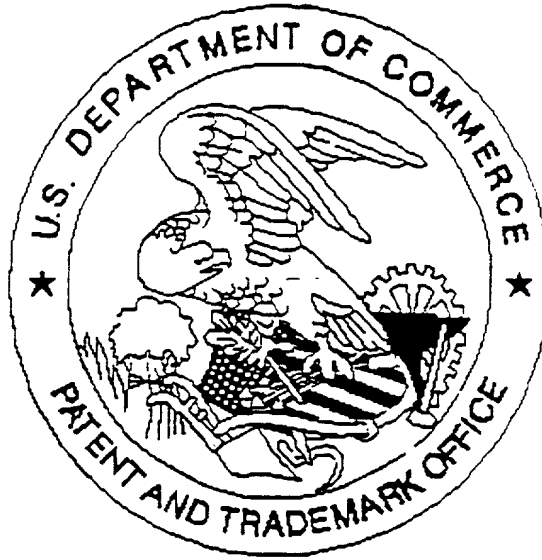
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